Structural Features of Pectin from *Hylocereus* Stem By-Products Revealed by NMR and Chromatographic Analysis

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ABSTRACT

Pectin is a structurally diverse plant polysaccharide widely used in food systems, yet detailed structural information from non-conventional plant sources remains limited. Despite the increasing interest in the valorization of dragon fruit by-products, the fine molecular structure of pectin isolated from *Hylocereus* stems has remained largely unexplored. In this work, pectin isolated from *Hylocereus* stem, an underutilized agricultural by-product, was comprehensively characterized using complementary spectroscopic and chromatographic techniques. One- and two-dimensional NMR spectroscopy (^{1}H, ^{13}C, COSY, HSQC, and HMBC), together with high-performance liquid chromatography and gel permeation chromatography, revealed a predominantly homogalacturonan backbone composed of repeating (→4)-α-D-galacturonic acid residues, with rhamnogalacturonan-I (RG-I) regions containing galactan and arabinan side chains. Molecular size distribution demonstrated a heterogeneous polymer population, while monosaccharide composition further supported the proposed structural architecture. This study provides detailed structural evidence for pectin from *Hylocereus* stems through the integrated use of complementary NMR and chromatographic analyses, expands current knowledge of pectins from underutilized agricultural biomass, and provides a structural basis for the future utilization of dragon fruit stem residues as sustainable sources of functional hydrocolloids and biopolymers.

KEYWORDS

Pectin, *Hylocereus* stem, NMR spectroscopy, Rhamnogalacturonan-I, Gel permeation chromatography

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INTRODUCTION

Pectin is a major structural polysaccharide in terrestrial plants, predominantly located in primary cell walls and the middle lamella, where it contributes to cell adhesion and wall integrity.[1,2] In addition to its biological role, pectin is widely used in food systems as a gelling, thickening, and stabilizing agent, and its functional properties are closely related to its molecular structure, degree of esterification, and branching pattern.[3-5] Structurally, pectin consists mainly of homogalacturonan (HG) regions formed by α-(1→4)-linked D-galacturonic acid residues and rhamnogalacturonan-I (RG-I) domains with repeating disaccharide units, often substituted with arabinan and galactan side chains.[6] The relative proportion and fine structure of these domains are known to vary depending on plant source, tissue type, and extraction conditions (Figure 1).[7]

Commercial pectin is commonly obtained from citrus peel and apple pomace, which remain the most extensively studied and industrially exploited sources.[8,9] Previous studies have indicated that pectin from these conventional materials differs in molecular weight distribution, degree of esterification, and side-chain composition, which in turn influence their functional properties.[10] In contrast, information on pectin derived from non-conventional plant materials is still limited, particularly with respect to detailed structural characterization using spectroscopic techniques such as NMR.[11] NMR spectroscopy is one of the most powerful analytical techniques for elucidating the fine structure of pectic polysaccharides, particularly in distinguishing homogalacturonan (HG) and rhamnogalacturonan-I (RG-I) domains, identifying glycosidic linkages, and characterizing substitution patterns.[12-15] More recently, increasing attention has been directed toward the recovery of pectin from non-conventional agricultural residues in response to the growing demand for sustainable biomass valorization. Green extraction technologies, including ultrasound-assisted, microwave-assisted, enzyme-assisted, and subcritical water extraction, have been widely investigated because they can improve extraction efficiency while preserving structural characteristics that govern pectin functionality.[16] At the same time, recent studies have emphasized that comprehensive structural characterization is essential for understanding the relationship between extraction conditions, molecular architecture, and functional performance.

Hylocereus spp. (dragon fruit) is widely cultivated in Vietnam and other tropical regions, generating substantial amounts of agricultural residues, including stems that are largely underutilized.[17] While pectin from fruit-derived materials has been reported, most previous studies have focused on extraction, physicochemical characterization, or biological activities, whereas detailed molecular structural investigations remain scarce. In particular, the fine structural organization of pectin isolated from *Hylocereus* stems, including the distribution of HG and RG-I domains and their branching characteristics, has not yet been comprehensively characterized using complementary NMR and chromatographic techniques.[18-20] This knowledge is important because subtle structural variations strongly influence the physicochemical properties and potential applications of pectin.

Based on the above considerations, the present study aimed to characterize pectin isolated from *Hylocereus* comprehensively by combining one- and two-dimensional NMR spectroscopy (^{1}H, ^{13}C, COSY, HSQC, and HMBC) with high-performance liquid chromatography (HPLC) and gel permeation chromatography (GPC). By integrating these complementary analytical techniques, this study provides detailed structural evidence for the molecular architecture of *Hylocereus* stems pectin, including its predominant HG backbone and RG-I domains with neutral sugar side chains. The results expand the current structural knowledge of pectin from non-conventional plant resources and provide a scientific basis for the valorization of dragon fruit stem residues as sustainable sources of functional hydrocolloids and biopolymers.

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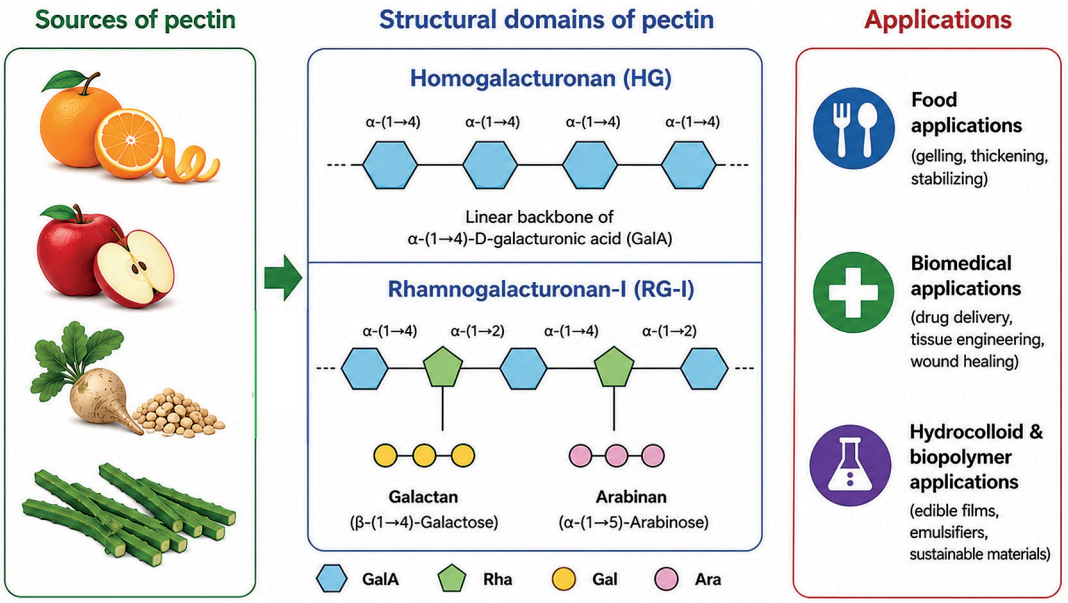


Figure 1. General structural domains, sources, and applications of pectin

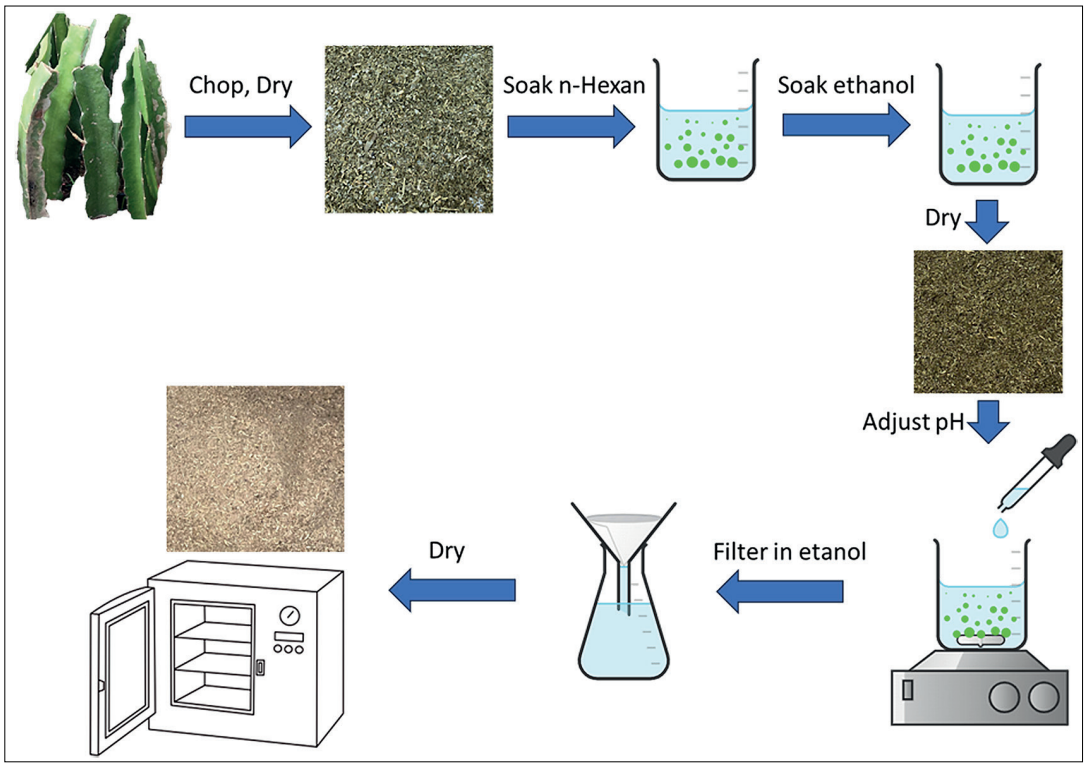


Figure 2. *Hylocereus* stems' pectin process

EXPERIMENTAL

Chemicals and Materials

Pectin was extracted from *Hylocereus* stems using the hot acid method with modifications (Figure 2).[21] Fresh dragon fruit stems were washed thoroughly to remove dirt and unwanted parts. The cleaned stems were chopped into small pieces, dried to a constant weight, and then coarsely ground. The powder was soaked in n-hexane (three times) at 40 °C for 3 hours, followed by filtration and drying of the residue. The residue was soaked with ethanol (three times) at 60 °C for 4 hours, filtered, and dried to obtain dragon fruit stem powder.

The powder was mixed with distilled water at a ratio of 1:30 (g/mL) and subjected to extraction using a heated stirring syringe at 80 °C for 120 minutes. During the stirring process, acetic acid was gradually added, and the pH of the mixture was monitored until it reached 3. Following 120 minutes of reaction, the pH was adjusted to 4–5 by the dropwise addition of 3.125 mol/L NaOH solution under constant stirring. Once the mixture cooled to room temperature, it was first filtered through a clean cloth, then sequentially filtered through 5-micron and 1-micron filters to obtain the dragon fruit stem extract.

Ethanol 96% was added to the extract at a ratio of 4:1 (mL/mL), followed by continuous stirring for 15 minutes and sedimentation to collect the precipitate. The precipitate was washed with 96% ethanol (4:1, mL/mL) three times until the recovered ethanol became clear. It was then washed with 70% ethanol (2:1, mL/mL) four times to remove remaining soluble compounds. A final wash with 96% ethanol was performed to reduce the solubility of pectin in water. The precipitate was then dried in an oven at 60 °C for 3 hours to obtain purified *Hylocereus* stamens' pectin.

Characterization using Fourier transform infrared spectroscopy (FTIR)

The characteristic functional groups of pectin were identified by fourier transform infrared spectroscopy (FTIR) within the wavenumber range of 400–4000 cm^{-1} using a Bruker ALPHA II FTIR spectrometer.

Determination of sugar content and composition

High-Performance Liquid Chromatography with Ultraviolet detection (HPLC-UV)

Determination of the neutral and acidic sugars in pectin was carried out by HPLC–UV after acid hydrolysis and PMP derivatization. Briefly, each pectin sample was hydrolyzed in an acidic medium, then reacted with 1-phenyl-3-methyl-5-pyrazolone (PMP) to form UV-active derivatives. The derivatized mixture was filtered and injected into an Agilent 1200 HPLC system equipped with a UV detector (DAD1 A, Sig = 245.8 nm, Ref = off).

Separation was achieved on a Zorbax Extend-C18 column (250 mm × 4.6 mm, 5 µm) maintained at 30 °C. An isocratic elution with a mobile phase of A (ammonium acetate buffer, pH 8.5) and B (acetonitrile) at 85:15 (v/v) was used. The flow rate was set to 1.0 mL/min, and a 10 µL aliquot of the PMP derivatized hydrolysate was injected.

Quantification of mannose, rhamnose, ribose, glucuronic acid, galacturonic acid, glucose, xylose, galactose, arabinose, and fucose was performed by relating peak heights to those of PMP derivatized standard solutions, using the calibration equation with a slope (m), intercept (b), and correlation coefficient of 1.00000: y = m x+b

Gel Permeation Chromatography (GPC)

The MWs of polysaccharides were determined by high-performance size-exclusion chromatography (HPSEC) using the HPLC instrument Shimadzu LC-20 Series (Shimadzu, Kyoto, Japan) equipped with an LC-20AD pump, degassing unit DGU-20A5R, autosampler SIL-20AHT, column oven CTO-20A, and refractive index detector RID-20A on the GPC column PSS SUPREMA combination ultrahigh (3 columns, dimensions of 8 mm x 300 mm, particle size of 10 µm, PSS, Mainz, Germany). Elution was carried out using 0.1 M lithium nitrate at 40 °C with a flow rate of 1.0 mL/min. As reference standards, several dextran standards (1, 5, 12, 25, 50, 150, 270, and 670 kDa) from Sigma (Cibolo, TX, USA) were employed.

Nuclear magnetic resonance spectroscopy (NMR) 1D and 2D spectroscopy

NMR spectra (1D and 2D) were recorded at 300 K on a Bruker spectrometer equipped with a 5 mm PABBO BB probe. Deuterium oxide (D_2O) was used as the solvent. ^{1}H NMR spectra were acquired at a resonance frequency of 500.241 MHz using the zgpr pulse program: acquisition time 2.04 s; number of scans 16; relaxation delay 2.0 s; spectral width 8012.82 Hz; 90° pulse width 10.20 µs. ^{13}C NMR spectra were recorded at a resonance frequency of 125.791 MHz using the zgpg30 pulse program: acquisition time 1.05 s; number of scans 256; relaxation delay 2.0 s; spectral width 31350.0 Hz; 90° pulse width 10.00 µs. Structural analysis was performed based on correlations observed in the 2D spectra, including: proton-proton correlations (COSY), direct ^{1}H–^{13}C correlations (HSQC) and long-range (2J, 3J) ^{1}H–^{13}C correlations (HMBC). All NMR spectra were processed using Bruker software with EM window function for 1D spectra and QSINE window function for 2D spectra. Since pectin is a heterogeneous polysaccharide rather than a single purified molecule, complete assignment of multiplicities and coupling constants for all proton resonances is not feasible due to extensive signal overlap. Therefore, structural characterization was performed by the combined interpretation of ^{1}H, ^{13}C, COSY, HSQC, and HMBC spectra, together with comparison with previously reported pectin NMR data.

RESULTS AND DISCUSSION

Fourier transform infrared spectroscopy (FTIR)

The FTIR spectrum of dragon fruit pectin is shown in Figure 3. The peak in the range of 3400–3300 cm^{-1} represents the stretching vibration of the -OH bond, present in galacturonic acid chains and monosaccharides such as rhamnose, arabinose, and galactose.[22] In general, the carboxylate ion stretching band (at 1631 cm^{-1}) was greater for the low degree of esterification (DM) pectin. Still, the ester carbonyl band (at 1748 cm^{-1}) was more intense for the high DE pectin.[23] FTIR reveals that *Hylocereus* stems contain high-methoxyl pectin, which was consistent with the DM determination (DM > 50%), because there is a prominent peak close to 1613 cm^{-1} and no absorption at about 1748 cm^{-1}, suggesting that methyl-esterified glucuronic acids were either absent or present in small amounts.

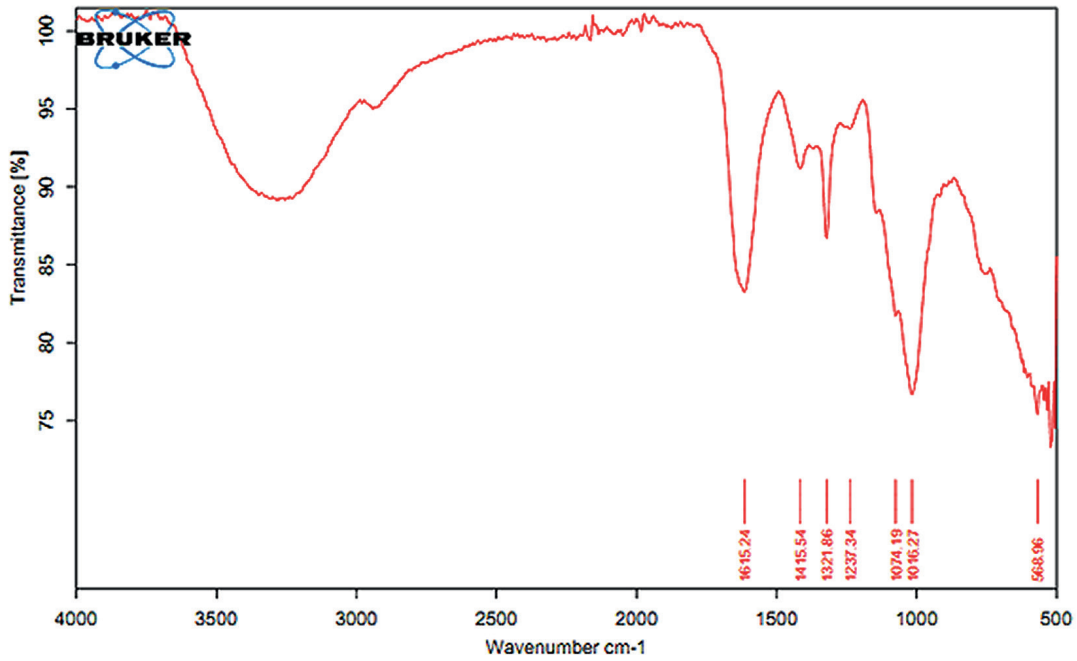


Figure 3. FTIR spectrum of *Hylocereus* stems' pectin

The peak at 1415 cm^{-1} is assigned to the symmetric stretching vibration of the -COO^- group and the bending vibration of the C-H group, which is also characteristic of deesterified galacturonic acid.[24] The absorption peak at 1327 cm^{-1} may be related to the bending vibration of the C-H group or the C-O stretching vibration of secondary hydroxyl groups, commonly found in rhamnose or galactose.[22] The vibration at 1237 cm^{-1} is due to the C-O bond in the ester group -$COOCH_3$ or acetyl group -$COCH_3$, reflecting the degree of methylation or acetylation of the galacturonic acid chain. The distinct peak at 1074 cm^{-1} is related to the C-O and C-O-C stretching vibrations in the glycosidic bond, which is characteristic of the polysaccharide structure.[22] In addition, the peak at 1016 cm^{-1} shows the pyranose ring vibration of the sugar units.[24]

Determination of sugar content and composition

Figure 4 shows the sugar composition in pectin. Glucose appears most abundantly with a content of up to 440.31 μg/mg, while the sugar content of Galacturonic acid in the cell walls of peach fruit accounts for the largest amount (about 64%).[25] Glucose accounted for the largest molar fraction (≈56.6%). This may reflect impurities (starch/oligo-glucans) or the presence of multiple glucose units in the side chains.

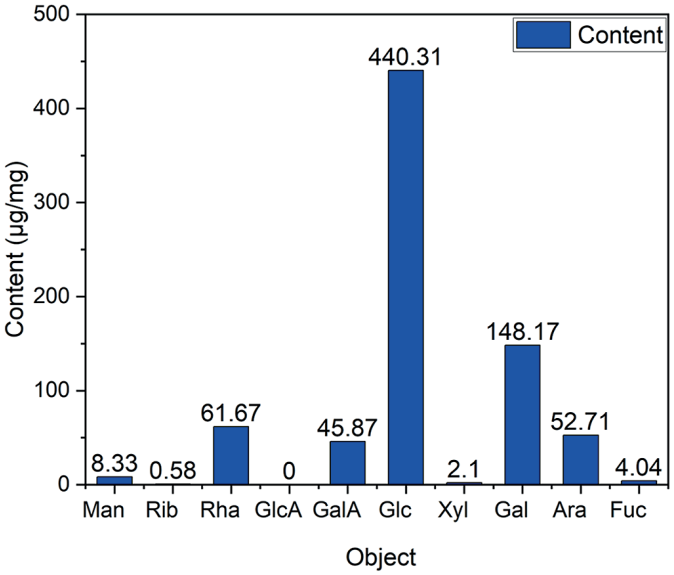


Figure 4. Sugar content of *Hylocereus* stems' pectin

In addition, galactose, rhamnose, arabinose, and galacturonic acid are also found to have decreasing contents. The presence of rhamnose, galactose, and arabinose confirms the RG-I region; galacturonic acid confirms the homogalacturonan backbone.[26] Mannose, fucose, xylose, and ribose, which are detected with low contents, less than 10 μg/mg, can be impurities of the extraction process. Glucuronic acid is not detected in the sugar composition, which is consistent with the FTIR results.

HPLC analysis reveals the monosaccharide composition of dragon fruit pectin (Figure 5). Distinct peaks were assigned to mannose (11.0 min), ribose (13.2 min), rhamnose (14.9 min), glucose (36.8 min), galacturonic acid (41.9 min), xylose (44.5 min), galactose (47.7 min), arabinose (51.2 min), and fucose (57.1 min). Glucose had the strongest peak among these, suggesting that it made up a large amount of the hydrolyzed combination.

While rhamnose, galactose, and arabinose are indicators of the rhamnogalacturonan-I (RG-I) domains, where rhamnose residues alternate with galacturonic acid and act as branching points for arabinan and galactan side chains, the presence of galacturonic acid clearly indicates the homogalacturonan backbone characteristic of pectin.[27] Since xylose, fucose, and mannose are typically small components in pectin, their detection indicates contributions from RG-II areas or co-extracted hemicellulosic polysaccharides.[28] Ribose is less frequent and could be co-purified with tiny carbs or trace contaminants from nucleotides. Combined with the monosaccharide content in Figure 3, *Hylocereus* stems' pectin is mainly RG-I domains.

Gel Permeation Chromatography (GPC)

The GPC results showed that the pectin sample had a fairly high average molecular weight (Mw ≈ 123 kDa) (Table 1). The dispersion (PDI = 1.42) confirmed that the sample had a relatively narrow size distribution, indicating that the polymer chains did not differ greatly in length, which is favorable for applications requiring uniformity in viscosity or gel formation. The highest molecular weight was nearly 668 kDa, indicating that the sample still contained long-chain pectin polymers that had not been shortened.

Table 1. Average molecular weights of *Hylocereus* stems' pectin

Parameter	Value (Da)
Number-average molecular weight (M_n)	86.629
Weight-average molecular weight (M_w)	122.679
Polydispersity index (M_w/M_n)	1.42
Max M_w	~667.800

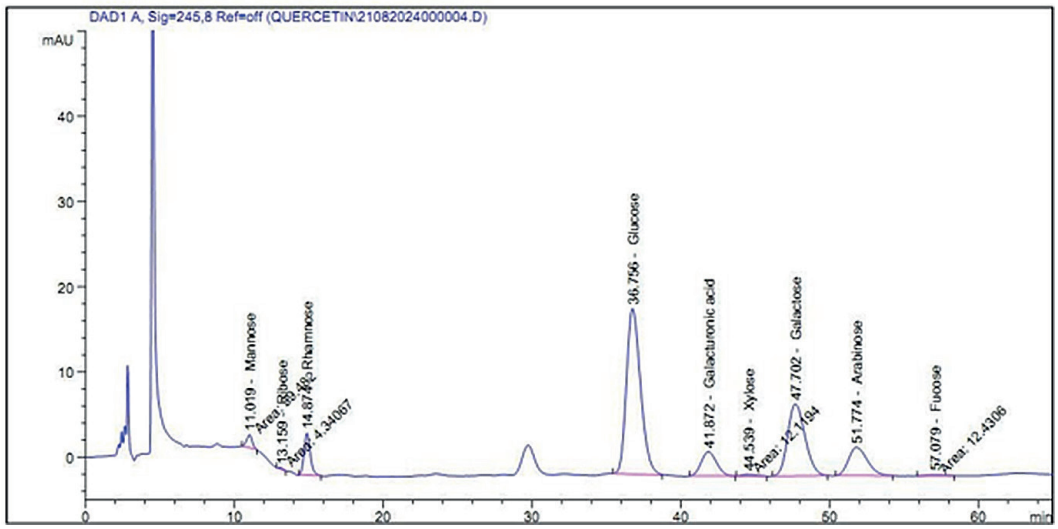


Figure 5. HPLC chromatogram of *Hylocereus* stems' pectin

Nuclear magnetic resonance spectroscopy (NMR) 1D and 2D spectroscopy

The ^{1}H and ^{13}C NMR spectra provided preliminary structural information on the isolated pectin in Figures 6 and 7. Resonance assignments were established through the combined interpretation of the ^{1}H, ^{13}C, COSY, HSQC, and HMBC spectra together with comparison to published NMR data for pectic polysaccharides. An anomeric proton resonance was observed at approximately δH 5.38 ppm, together with characteristic signals in the anomeric carbon region of the ^{13}C spectrum. These resonances are consistent with the presence of α-linked galacturonic acid residues commonly reported for homogalacturonan-rich pectins. Because resonance overlap is common in polysaccharides, the assignments were established from the combined interpretation of the available 2D NMR spectra rather than from a single correlation.

The COSY spectrum was used primarily to identify proton spin systems and sequential connectivities within the carbohydrate residues, providing complementary information for resonance assignment (Figure 8). The HSQC spectrum provided one-bond ^{1}H–^{13}C correlations, whereas the HMBC spectrum supplied longer-range heteronuclear correlations that assisted the structural interpretation (Figure 9).

The signals of ^{1}H NMR in the range from 3.35–4.59 ppm were from ring protons, while ring carbons in ^{13}C NMR are from 69–80 ppm. C-6 signals (H/C: 3.54; 3.46/ 62.38; 60.15) are priority assignment to CH_2-O in Glc and Gal. In the 2.7–4.2 region, they are mainly the CH(-O) proton of GalA, Glc, Gal, or Rha. The proposed resonance assignments are summarized in Table 2 and Figure 10. Owing to partial signal overlap commonly observed in polysaccharide NMR spectra, several assignments should be regarded as tentative and were established from the combined interpretation of the available spectroscopic data.

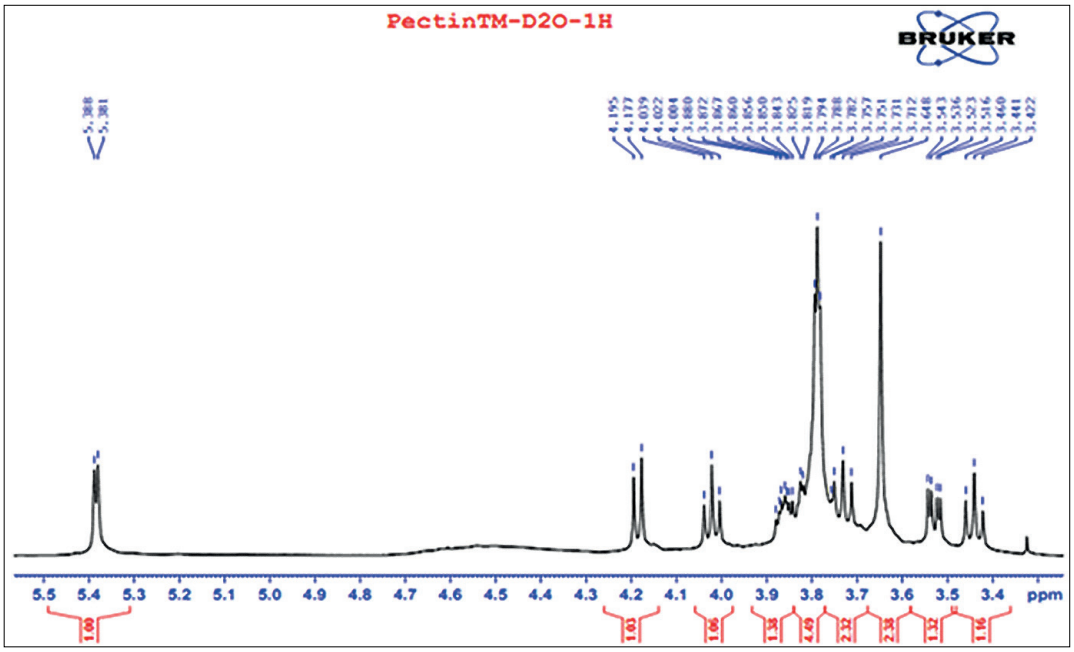


Figure 6. ^{1}H NMR spectrum of *Hylocereus* stems'pectin

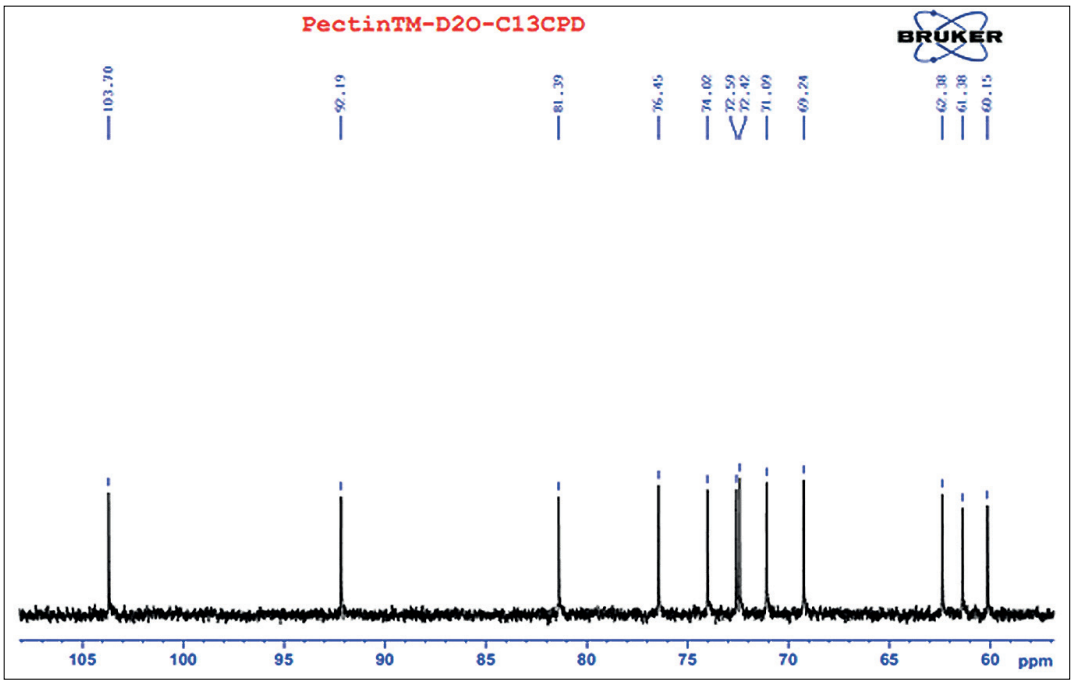


Figure 7. ^{13}C NMR spectrum of *Hylocereus* stems' pectin

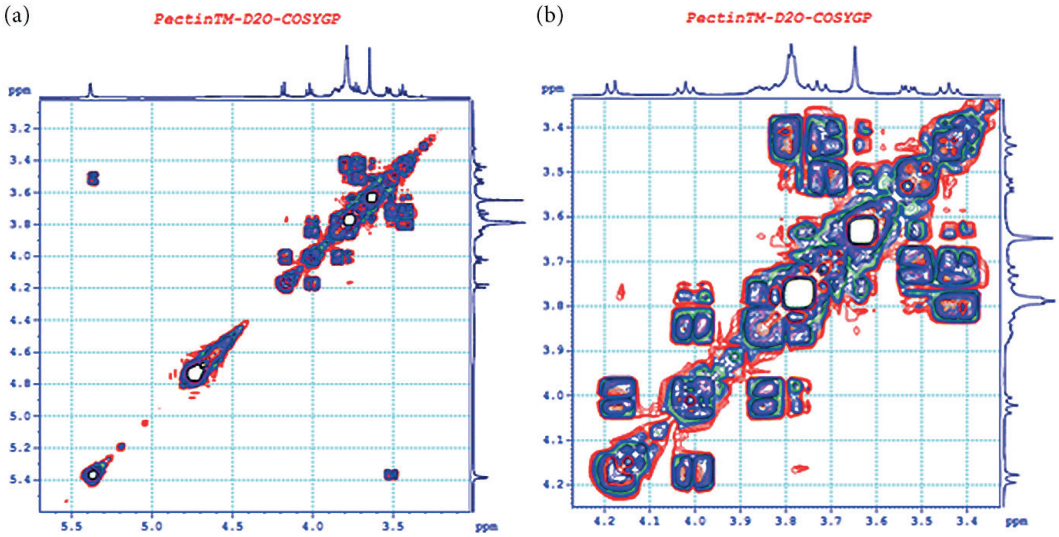


Figure 8. COSY spectrum of *Hylocereus* stems' pectin

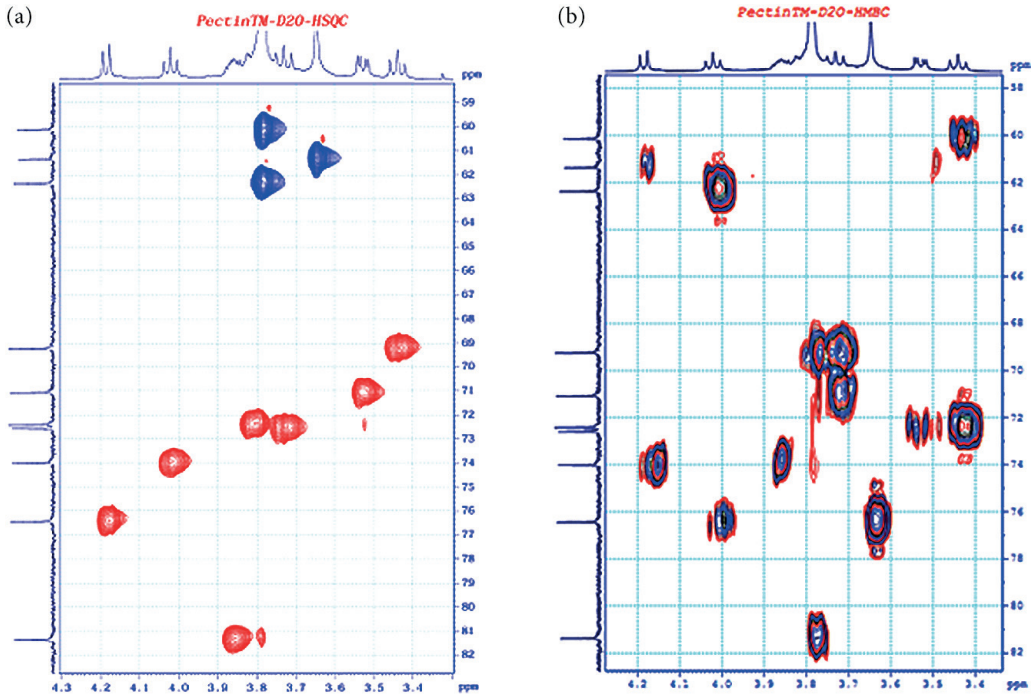


Figure 9. (a) HSQC and (b) HMBC of dragon fruit pectin

Assignments were established from the combined interpretation of ^{1}H, ^{13}C, COSY, HSQC, and HMBC spectra together with comparison to previously reported chemical shifts for pectic polysaccharides. Owing to signal overlap commonly observed in polysaccharide NMR spectra, several assignments should be regarded as tentative.

Structural prediction

The monosaccharide composition analysis revealed that the polysaccharide was mainly composed of galacturonic acid, rhamnose, galactose, and arabinose, indicating that the isolated polysaccharide belongs to the pectic family. In addition, the NMR spectra demonstrated characteristic signals corresponding to α-linked galacturonic acid residues and rhamnose units, together with signals attributed to arabinose and galactose residues. These results suggest that the polysaccharide contains both homogalacturonan (HG) and rhamnogalacturonan-I (RG-I) structural domains. Based on the combined evidence from monosaccharide composition and spectroscopic analysis, a tentative structural model of the pectin was proposed, as illustrated in Figure 11.

Based on the monosaccharide composition and NMR analysis, the pectin isolated from *Hylocereus* stems is proposed to consist mainly of homogalacturonan (HG) and rhamnogalacturonan-I (RG-I) domains. The backbone is predominantly composed of →4)-α-D-GalpA-(1→ residues, forming linear HG regions represented by consecutive GalA units in the schematic model. In addition, the occurrence of rhamnose residues indicates the presence of RG-I segments characterized by the repeating disaccharide motif →4)-α-D-GalpA-(1→2)-α-L-Rhap-(1→. These RG-I regions act as branching points where side chains are attached. As illustrated in the proposed structure, the side chains mainly consist of arabinan and galactan, represented by short chains of α-(1→5)-linked arabinose residues and longer chains of β-(1→4)-linked galactose residues, respectively. Polysaccharide is a typical pectic polysaccharide dominated by HG regions with branched RG-I domains.

Table 2. Assigned peak prediction

No	δH (ppm)	δC (ppm)	Observed signal	Proposed monosaccharide assignment	Basis of assignment
1	5.38	103.7	Anomeric proton/carbon signal	Galacturonic acid (GalA)	Tentatively assigned based on the combined interpretation of the ^{1}H, COSY, HSQC, and HMBC spectra together with characteristic chemical shifts of GalA reported in previous studies. The assignment is also consistent with the HPLC monosaccharide composition.
2	4.19	76.45	Ring proton/carbon signal	C-4 GalA	Assignment supported by the characteristic downfield C-4 chemical shift together with HMBC correlation and literature comparison for α-(1→4)-linked GalA residues.
3	4.03	74.02	Ring proton/carbon signal	H-2 GalA or Gal/Glc	Tentatively assigned to H-2/C-2 of GalA based on COSY connectivity from the anomeric proton and agreement with published chemical shift data.
4	3.87	81.4	Ring proton/carbon signal	H-3 GalA	Assigned according to the combined interpretation of the 2D NMR spectra and comparison with published assignments for homogalacturonan structures.
5	3.79 and 3.78	71.09/72.42	Overlapping ring proton/carbon signals	H-5 GalA or H-4, H-5 Gal/Glc overlap	Assigned to overlapping ring proton/carbon resonances commonly observed in pectic polysaccharides.
6	3.54	62.38	Methylene proton/carbon signal	Glc/Gal (C-6, CH_2-O)	Consistent with C-6 methylene resonances of hexopyranose residues (Glc/Gal) reported in the literature.
7	3.46	60.15	Methylene proton/carbon signal	H-6β/C-6 Glc/Gal	Assigned as the second methylene proton of C-6 based on its characteristic chemical shift and the neighbouring methylene resonance.
8	Cluster 3.51	69-74	H-ring	Ring protons from Glc/Gal	Broad overlapping resonances within the typical carbohydrate region were assigned from combined 2D NMR interpretation and supported by the HPLC monosaccharide profile.

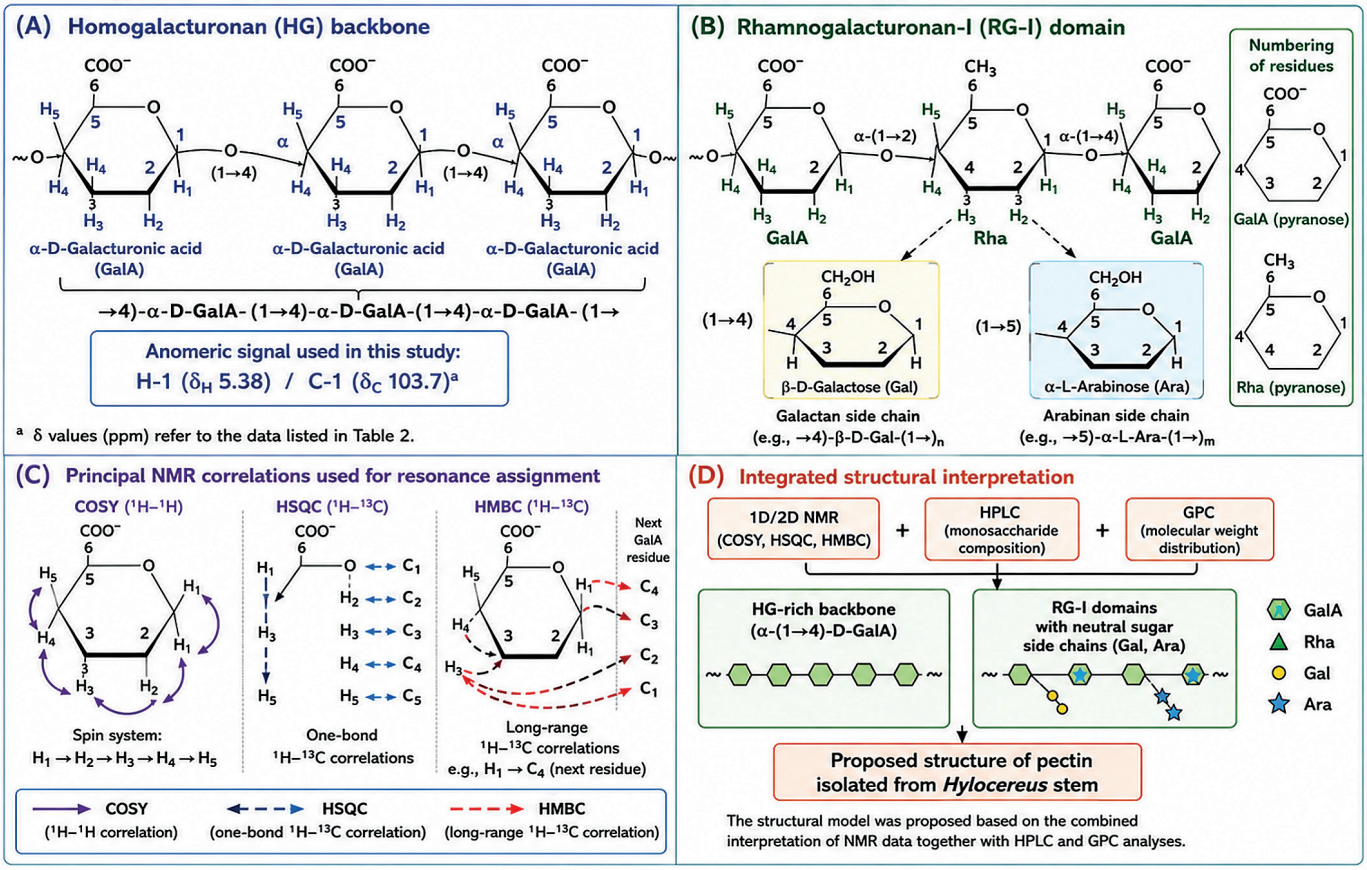


Figure 10. Proposed structural model and NMR assignment strategy of *Hylocereus* stems' pectin

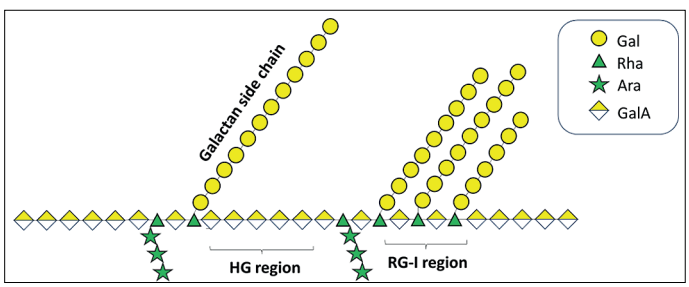


Figure 11. Proposal structure of *Hylocereus* stem's pectin

CONCLUSION

Hylocereus stems' pectin was comprehensively characterized using complementary NMR spectroscopy, HPLC, and GPC analyses. Integrated interpretation of ^{1}H, ^{13}C, COSY, HSQC, and HMBC spectra demonstrated that the polymer is predominantly composed of a homogalacturonan (HG) backbone consisting of (→4)-α-D-galacturonic acid residues, together with rhamnogalacturonan-I (RG-I) domains containing galactose- and arabinose-rich side chains. HPLC analysis following acid hydrolysis confirmed galacturonic acid, rhamnose, galactose, and arabinose as the principal monosaccharide constituents, supporting the proposed structural assignments. The relatively high glucose content is most likely attributable to co-extracted glucan- or starch-derived components, although limited incorporation into the pectic fraction cannot be completely excluded. GPC analysis indicated an average molecular weight (Mw) of approximately 122.7 kDa, a number-average molecular weight (Mn) of 86.6 kDa, and a polydispersity index (PDI) of 1.42, suggesting a relatively homogeneous polymer population. Overall, this study provides comprehensive structural evidence for pectin isolated from the underutilized *Hylocereus* stems and expands the current knowledge of pectin from non-conventional plant resources, thereby supporting the valorization of dragon fruit agricultural residues as a potential source of natural hydrocolloids and biopolymers. Future studies should investigate the relationship between the molecular structure and the physicochemical, rheological, and biological properties of this pectin further to evaluate its potential in food and biomedical applications.

SUPPLEMENTARY INFORMATION

There is no Supplementary Information for this article.

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AUTHOR CONTRIBUTIONS

Phuong-Chien Tran: Investigation, Formal analysis, Writing original draft, Conceptualization. Anh-Kien Le: Supervision, Review & editing. Thi-Hai-Yen Nguyen: Writing original draft, Formal analysis. Thi-Kim-Dung Hoang: Supervision, Review & editing, Funding acquisition

DECLARATION OF COMPETING/FINANCIAL INTERESTS

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

DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES

During the preparation of this manuscript, ChatGPT (OpenAI, GPT-5.5 Free version) was used solely to improve grammar, language clarity, and readability. All scientific content, data interpretation, and conclusions were developed and verified by the authors. The authors take full responsibility for the final content of the manuscript.

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