

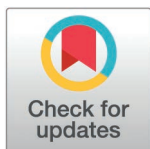
RESEARCH ARTICLE

Structural characterization and antifungal properties of sequentially extracted polysaccharides from Algerian *Opuntia ficus-indica* L. cladodes

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Abstract

Opuntia ficus-indica L., a xerophytic cactus native to arid regions, is an understudied source of structurally varied polysaccharides with potential for bioactivity. Four different types of substances were separated in this study, which is the first time this method has been used for Algerian varieties: cellulose (19.1%), pectins (7.20%), hemicelluloses (2.57%), and water-soluble mucilage (8.84% of dry mass). Pectins rich in homogalacturonans (33.5% galacturonic acid) and rhamnogalacturonans (30% rhamnose) were identified by monosaccharide profiling, along with hemicelluloses that were dominated by xylose (55.9%). FTIR spectroscopy validated structural signatures, such as β -glycosidic linkages (890 cm^{-1}) and carboxylate groups (1750 cm^{-1}) in pectins. *Penicillium* sp. was shown to be dose-dependently inhibited by antifungal assays, with a 75% growth reduction at 100 mg/mL pectin concentration ($*p < 0.05$ vs control). The structure of polysaccharides, especially the amount of carboxylate, is correlated with the observed bioactivity. Based on their biocompatibility and regional adaptability, these results establish Algerian *O. ficus-indica* polysaccharides as viable options for pharmaceutical and food preservation applications.

1. Introduction

Because of their biocompatibility and structural diversity, plant-derived polysaccharides have become sustainable biomaterials with a wide range of uses, especially in pharmaceuticals and food preservation [1]. These include the CAM-adapted xerophyte *Opuntia ficus-indica* L., which yields cladodes rich in hemicelluloses (xylans), pectins

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Data availability statement: All raw data are available on Figshare: <https://doi.org/10.6084/m9.figshare.29508983.v4> including: - FTIR spectra - GC monosaccharide quantification - Antifungal assay measurements - A detailed README.txt file explaining file organization and methods.

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(rich in rhamnogalacturonan), and mucilage (water-soluble polysaccharides), the composition of which varies greatly depending on the region of origin [2,3]. Despite their potential for producing high-value polysaccharides, Algerian ecotypes, exposed to particular arid conditions, remain unexplored, in contrast to Mexican and Mediterranean cultivars that have been thoroughly studied [4].

Recent advances have highlighted the importance of structure-function relationships in polysaccharide bioactivity. For instance, xylose-rich hemicelluloses from Agave exhibit antifungal properties via membrane disruption [5], while RG-I pectins from Aloe vera inhibit *Aspergillus* through carboxylate-mediated interactions [6].

However, despite this progress, systematic comparisons of bioactivity across sequentially extracted fractions (e.g., mucilage vs. pectins) from *Opuntia ficus-indica* L. cladodes are lacking. Moreover, the potential of Algerian ecotypes, which may produce polysaccharides with unique structural features and enhanced bioactivity due to their environmental adaptations, is particularly underexplored.

This study bridges critical knowledge gaps by developing the first sequential extraction protocol specifically optimized for Algerian *Opuntia ficus-indica* L. cladodes, enabling concurrent isolation of four native polysaccharide classes: mucilage (8.84%), pectins (7.20%), hemicelluloses (2.57%), and cellulose (19.1%). Through integrated GC and FTIR analyses, we reveal distinctive structural signatures including xylose-dominated hemicelluloses (55.9%) and pectins rich in galacturonic acid (33.5%) and rhamnose (30%). Importantly, we demonstrate dose-dependent antifungal activity against *Penicillium* sp., with bioactivity strongly correlated to carboxylate abundance (FTIR 1750 cm⁻¹, R²=0.89). These findings establish the first structure-function framework for Algerian cultivars, highlighting their potential as sustainable natural preservatives for food applications in arid regions.

2. Materials and methods

2.1. Water content determination

Cladodes were harvested in November from approximately 10-year-old *Opuntia ficus-indica* L. plants growing under natural conditions at the University of Science and Technology of Oran Mohamed-Boudiaf, Algeria (35°44'N; 0°33'W). A single cladode per plant was used for the entire extraction process to ensure biological consistency.

The cladode was cut into small fragments (≈5×5 mm), and the fresh mass (FM) was immediately measured. The fragments were frozen at -80°C and then lyophilized for 5 days until a constant dry mass (DM) was achieved.

The water content (WC) estimated in percentage is calculated using the following formula:

$$WC = \frac{FM - DM}{FM} \times 100$$

The resulting dry material was ground into a fine powder using a liquid nitrogen-cooled mill and stored in airtight containers at -20°C until extraction.

2.2. Sequential extraction of polysaccharides from *Opuntia ficus-indica* L

A sequential extraction protocol was performed on the defatted cladode powder of *Opuntia ficus-indica* L. to isolate mucilage, pectins, hemicelluloses, and cellulose, based on methodologies from Habibi et al. (2004) and Chaa et al. (2008) [2,7]. The overall process is summarized in Fig 1 (mucilage extraction) and Fig 2 (pectin, hemicellulose, and cellulose extraction).

Defatting. The dry powder was first defatted using a toluene-ethanol mixture (38:62, v/v) for 24 hours at room temperature to remove lipophilic compounds. The defatted powder was collected by filtration and air-dried prior to polysaccharide extraction.

Mucilage extraction. The defatted powder was subjected to three consecutive aqueous extractions using distilled water at 20°C for 2 hours per step (1:20 w/v ratio). Each extraction was performed on the residual solid from the previous step to sequentially recover water-soluble polysaccharides. After each extraction, the supernatant was collected by centrifugation (4000 rpm, 15 min). The mucilage from each supernatant was precipitated with absolute ethanol (1:3 v/v) ratio (one volume of aqueous extract to three volumes of ethanol), yielding three fractions: water-soluble mucilage M1 (from the first supernatant), M2 (from the second supernatant), and M3 (from the third supernatant). The remaining pellet after the third extraction was designated as the demucilaged powder (Residue R1).

Pectin extraction. Pectins were extracted in two sequential steps from the demucilaged powder (R1).

- **Highly esterified pectins (P1):** R1 was treated with hot distilled water at 80°C for 2 hours (1:20 w/v ratio). The extract was concentrated under reduced pressure, precipitated with absolute ethanol (1:3 v/v) ratio for 24 hours at 4°C, and centrifuged (3900 rpm, 20 min). The resulting pellet was designated as Pectin 1 (P1).
- **Lowly esterified pectins (P2):** The residue from the hot water extraction was subsequently treated with a 1% (w/v) EDTA solution (pH 6.5) for 4 hours at 80°C (1:20 w/v ratio). The resulting extract was dialyzed (MWCO 6–8 kDa) against distilled water for 3 days, precipitated with ethanol at 3:1 (v/v) ratio for 24 hours at 4°C, and centrifuged (3900 rpm, 1 hour). The final pellet was designated as Pectin 2 (P2).

Lignin removal. The residue from the EDTA extraction was treated with a 1% NaOH solution prepared in 75% ethanol for 2 hours at 80°C to solubilize lignins [8].

Hemicellulose and cellulose extraction. The delignified residue was treated with a 14% (w/v) KOH solution for 14 hours at room temperature under agitation (1:20 w/v ratio). After centrifugation (4000 rpm, 20 min), the supernatant (alkali-soluble fraction) was acidified to pH 5.8 with glacial acetic acid, causing the hemicellulose fraction (H) to precipitate. The precipitate was recovered by centrifugation, dialyzed (MWCO 6–8 kDa) against distilled water for 5 days to remove residual salts, and lyophilized. The final insoluble residue after KOH treatment was considered the cellulose-rich fraction (C).

All extracted fractions were neutralized (where applicable), dialyzed against distilled water, lyophilized, and stored at –20°C until further analysis.

2.3. Sugar assay by colorimetry

Sugar assays are performed using sulfuric-acid phenol method [9] for neutral sugars and meta-hydroxy diphenyl (m-HDP) method for uronic acids [10]. Due to the interference of uronic acids in the determination of neutral sugars and vice versa, a correction method established by Montreuil and Spik (1963) [11] must be applied.

2.4. Fourier Transform Infrared Spectroscopy (FTIR) analysis

The structural characteristics of the extracted polysaccharide fractions from *Opuntia ficus-indica* L. cladodes were investigated using Fourier Transform Infrared (FTIR) spectroscopy. The analysis was carried out on a FTIR spectrometer in

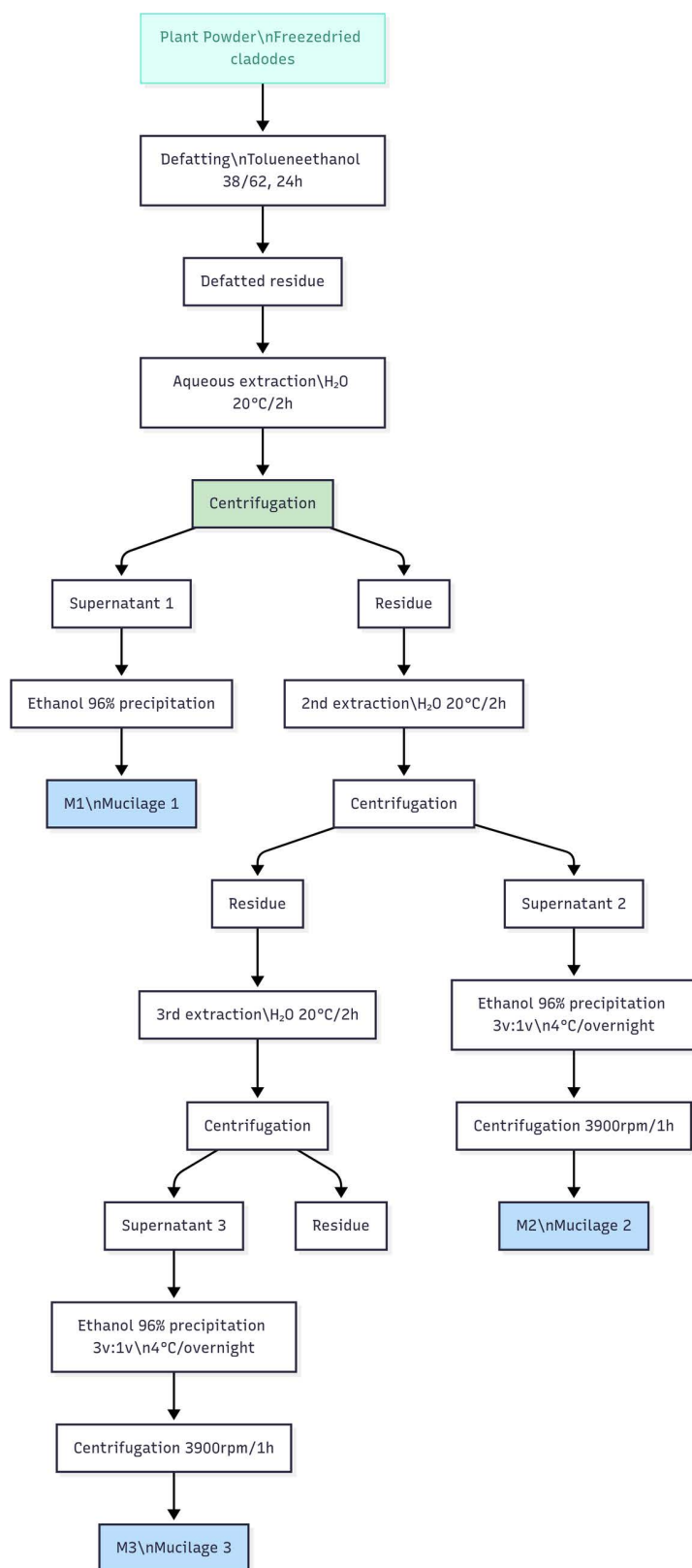


Fig 1. Modified Mucilage Extraction Protocol [2].

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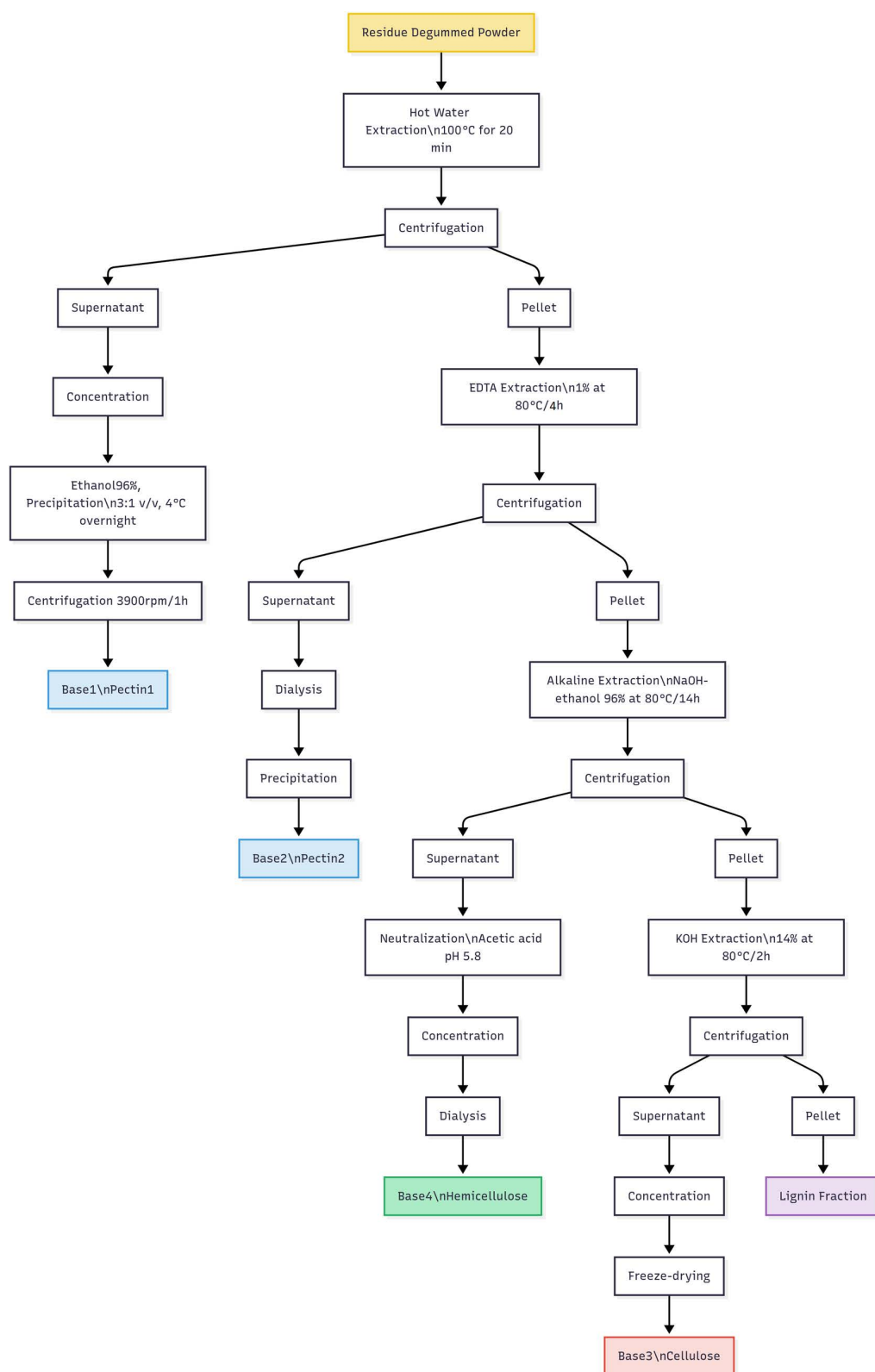


Fig 2. Parietal Molecules Extraction Protocol (Pectins, Celluloses and Hemicelluloses) Cladodes Opuntia ficus indica L. [7].

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attenuated total reflectance (ATR) mode, without the need for KBr pellet preparation. Each sample was scanned over a wavelength range of 4000–400 cm^{-1} at a resolution of 4 cm^{-1} , with 32 scans per measurement to ensure signal-to-noise ratio improvement.

The resulting spectra were analyzed using OMNIC™ Software (Thermo Fisher Scientific) to identify characteristic absorption bands corresponding to specific functional groups and glycosidic linkages present in different types of polysaccharides, including pectins, hemicelluloses, cellulose, and mucilages. Key spectral features such as O–H stretching vibrations ($\sim 3400 \text{ cm}^{-1}$), C–H stretching ($\sim 2920 \text{ cm}^{-1}$), carboxylic acid groups ($\sim 1750 \text{ cm}^{-1}$), and glycosidic linkages in the “sugar region” ($1200\text{--}950 \text{ cm}^{-1}$) were used to determine the chemical nature and structural differences among the extracted fractions.

The FTIR results were further correlated with monosaccharide composition data obtained by gas chromatography (GC) to validate the structural assignments and to support the interpretation of the biological activity observed in this study.

2.5. monosaccharide composition analysis by GC

Monosaccharide composition was determined by gas chromatography (GC) using a Shimadzu GC-2030 system equipped with a DB-5 capillary column (30 m length $\times$ 0.25 mm internal diameter $\times$ 0.25 μm film thickness). The temperature program was as follows: initial hold at 160°C for 2 min, ramp to 250°C at 5°C/min, and final hold at 250°C for 10 min. Samples were derivatized to trimethylsilyl ethers using pyridine/hexamethyldisilazane/trimethylchlorosilane (10:2:1, v/v/v) at 80°C for 30 min. Derivatives were detected by flame ionization detector (FID) at 280°C, with helium as carrier gas (flow rate: 1 mL/min). Calibration was performed using external standards (arabinose, rhamnose, xylose, galactose, glucose, mannose, glucuronic acid, and galacturonic acid).

2.6. Biological activity of pectins and hemicelluloses extracted from *O. ficus-indica* L. Against *Penicillium* sp

To assess the antifungal activity, we prepared Potato Dextrose Agar (PDA) media where the dextrose content was partially or completely replaced by the extracted polysaccharides. For each polysaccharide type (pectin and hemicellulose), we prepared a series of five PDA media with varying dextrose/polysaccharide ratios (100/0, 75/25, 50/50, 25/75, and 0/100), as detailed in Table 1. This design allowed us to test a concentration-dependent effect of the polysaccharides on fungal growth.

All prepared culture media were sterilized by autoclaving at 121°C for 20 minutes. Subsequently, a 5 mm mycelial plug was aseptically excised from the peripheral growing edge of a fresh *Penicillium* sp. culture and inoculated in the center of each Petri dish containing the amended media (Table 1). The radial growth of the fungus was monitored daily for 10 days by measuring the diameter of the mycelial colony. The percentage inhibition of mycelial growth (PIMG) for each treatment was calculated relative to the untreated control using the following formula:

$$\text{Percentage Reduction in Growth} = 100 \times \left(\frac{X - Y}{X} \right)$$

Where:

- X = Growth of the pathogen alone without antagonist (control).
- Y = Growth of the pathogen with the antagonist.

Table 1. Dextrose/Pectin and dextrose/hemicellulose ratio in PDA Media.

Dextrose %	100	75	50	25	0
Pectin %	0	25	50	75	100
Hemicelluloses %	0	25	50	75	100

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Each replicate originated from separate cladode collections harvested under standardized conditions: samples were taken in November from adult plants (approximately 10 years old) growing in the same location. A single cladode per plant was used to ensure biological consistency.

2.7. Statistical analysis

Three independent biological replicates ($n=3$) were analyzed for each experiment.

All experiments were performed in triplicate ($n=3$ independent biological replicates). Data are expressed as mean $\pm$ standard deviation (SD). Statistical analyses were conducted using:

- **GraphPad Prism** (GraphPad Software, USA) for **ANOVA**, correlation, and dose-response modeling.
- **R v4.3.1** (packages lme4 for mixed models, car for ANOVA assumptions) for supplementary validation.

Assumptions and corrections.

- Normality was confirmed via **Shapiro-Wilk test** ($p > 0.05$). Non-normal data were log10-transformed.
- Multiple comparisons were adjusted using **Bonferroni correction** (family-wise $\alpha = 0.05$).
- Post-hoc power analysis (**GPower**, $\alpha = 0.05$, effect size $f = 0.8$) confirmed $>80\%$ power for key comparisons (e.g., 75% inhibition).
- **Outliers (>3 SD)** were identified via Tukey's method and excluded ($n = 2/120$ datasets). Sensitivity analyses confirmed robustness of results with/without outliers.

Significance threshold was set at $p < 0.05$. Full statistical outputs (F/t-values, degrees of freedom, exact p-values) are provided in Supplementary S1 File.

3. Results and discussion

3.1. Water content

The water content in *Opuntia ficus-indica* L. cladodes was 91.80%. This content can be explained by the adaptive strategy of cacti. Indeed, this species is a xerophytic plant belonging to CAM plants, which grows in arid and semi-arid climates. [12].

3.2. Yield and colorimetric assay of extracted fractions

The extraction sequence adopted for polysaccharides from *Opuntia ficus-indica* L. cladodes allowed us to divide them into four main groups: mucilage, pectin, hemicelluloses, and cellulose. Table 2 summarizes the yields obtained and the quantities of different polysaccharides extracted from *Opuntia ficus-indica* L. cladodes.

The four main polysaccharide groups from prickly pear cladodes were obtained in different quantities;

The mucilage is recovered with a yield of 8.84% relative to the total dry matter; this result is comparable to that obtained for the cladodes of *Opuntia ficus-indica* L. with a mass yield of 6% [13] and a yield of 22.96% compared to all the polysaccharides. Note that the yield of mucilage is lower than those obtained during extractions of mucilages from dry cladodes of cactaceae such as *Opuntia* sp., and *Opuntia joconostle* and *C. triangularis*; where the yields described were 18%, 30%, and 26.6%, respectively. [3,14,15]

The pectic fraction is 7.2% compared to the dry matter and 18.7% compared to the polysaccharide material; it is made up of two sub-fractions successively extracted with water and EDTA which represents 10.2% and 89.03% compared to the entire pectic fraction.

Table 2. Mass yield and colorimetric dosage of the fractions extracted from the different fractions of *Opuntia ficus-indica* L cladodes.

Fraction	Yield (% dry mass)	Neutral Sugars [ON] (%)	Uronic Acids [AU] (%)	Significance (p<0.05)
Mucilage 1 (M1)	7.34±0.45a	85.8±2.1a	14.2±1.8a	a vs b, c
Mucilage 2 (M2)	0.80±0.12b	89.0±1.7a	11.0±1.5a	b vs a, d
Mucilage 3 (M3)	0.70±0.10b	60.3±3.2b	39.7±2.9b	b vs a, c
Pectin 1 (P1)	0.79±0.15b	66.4±2.8c	33.6±2.5c	c vs a, d
Pectin 2 (P2)	6.41±0.50c	62.0±2.3c	38.0±2.1c	c vs a, b
Hemicellulose (H)	2.57±0.30d	95.1±1.5d	4.9±0.9d	d vs all
Cellulose (C)	19.1±1.2e	100±0.0e	0±0.0e	e vs all
Lignin (L)	0.78±0.08b	—	—	b vs a, e

-M1: mucilage1, M2: mucilage2, M3: mucilage3, P1: pectin1, P2: pectine2, H: Hemicelluloses, C: cellulose, L: lignins, ON: Neutral oses, AU: Uronic acids

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This can be explained by the presence of covalent bonds which link the pectins to the other parietal constituents [16], where they form more or less strong bonds on the one hand between them (rhamnogalacturonans II), and on the other hand with other cell wall molecules (hemicelluloses, lignins, suberins), and which makes their extractions difficult. [17]

The cellulosic fraction is the largest, 19.1% compared to the initial dry matter, which corresponds to 49.62% of the total cell wall polysaccharide in comparisons with the work of Melainine (2003) [18], on other cacti, where the yield was $15.0 \pm 6.7\%$ for *Opuntia ficus-indica* L., compared to $11.5 \pm 7.8\%$ for *Cereus jamacaru*.

The fraction of hemicelluloses is very low compared to the other fractions, 2.57% compared to the dry matter which corresponds to 6.64% of all the extracted fractions. Hemicelluloses are in lower proportion in the secondary walls and more difficult to extract in the secondary tissues.

The polysaccharides of mucilage1 are composed of 85.8% neutral oses and 14.2% uronic acid, and 89% neutral oses, 11% uronic acids for mucilage2 against 60.3% neutral oses and 39.7% acids uronics for mucilage3. As widely described in the literature, the chemical composition and yield of extractions of water-soluble polysaccharides from cactaceae cladodes vary according to various conditions such as climatic environment, location, geographical origin, and harvest period [3].

The pectic fraction extracted with hot water is made up of 66.4% neutral oses and 33.6% uronic acids; on the other hand, the pectic fraction extracted by EDTA is composed of 62% neutral oses and 38% uronic acids. Uronic acid; the hemicellulosic fraction is composed of 95.1% neutral ose, and 4.9% uronic acids, and the cellulose fraction is 100% neutral ose, which explains the effectiveness of the extraction protocol.

3.3. Structural Characterization by Fourier Transform Infrared Spectroscopy (FTIR)

We employed Fourier-transform infrared (FTIR) spectroscopy to examine the structural characteristics of polysaccharide fractions derived from *Opuntia ficus-indica* L. cladodes. The approach identified specific functional groups in each fraction, enabling the differentiation of pectins, hemicelluloses, cellulose, and mucilages according to their unique vibrational signatures.

All fractions exhibited a pronounced, extensive absorption band at about 3400 cm^{-1} , indicative of O-H stretching vibrations in strongly hydrogen-bonded polysaccharides. A pronounced peak near 2920 cm^{-1} was noted in all spectra, indicative of asymmetric C-H stretching from methylene (CH_2) groups within sugar rings.

In the carbohydrate fingerprint region ($1200\text{--}950 \text{ cm}^{-1}$), several clear peaks appeared, showing C-O-C and C-O-H stretching and bending vibrations. These bands furnish essential structural information, elucidating glycosidic linking patterns and the anomeric configurations of the constituent monosaccharides.

Specific fraction characterization:.

a. Pectin Fractions (P1 and P2)

The FTIR spectra of the pectin fractions (P1 and P2) showed a distinct peak at $\sim 1750\text{ cm}^{-1}$, assigned to the carbonyl group (C=O) of carboxylic acid moieties (Fig 3). This band is a hallmark of acidic pectins containing galacturonic acid residues. Additionally, a peak at approximately $\sim 890\text{ cm}^{-1}$ suggests the presence of β -glycosidic linkages, often found in linear pectins such as homogalacturonans.

The spectral evolution clearly demonstrates enrichment of carboxylated pectins (increasing C=O peak) and reduction of interference (decreased background noise), confirming the efficacy of sequential extraction. These observations corroborate GC data showing increased galacturonic acid content (Table 4).

The C=O peak intensity, maximal in P2, correlates with enhanced antifungal activity (Section 3.5), suggesting that free carboxyl groups in pectins may interact with fungal proteins through electrostatic or hydrogen bonding mechanisms.

b. Hemicellulose

The hemicellulosic fraction had spectral traits that were like pectins', but with some important differences:

- The $\sim 3400\text{ cm}^{-1}$ band was also there, which means there are a lot of hydroxyl groups.
- More peaks were seen between 1200 and 950 cm^{-1} , which is consistent with the presence of pentoses like xylose and separates hemicelluloses from other parts.

c. Cellulose

The spectrum of cellulose was more regular than those of the other parts. It had a simpler profile in the 1200 – 950 cm^{-1} range, which fits with its highly organised crystalline structure. The O–H stretching band at about 3400 cm^{-1} was still

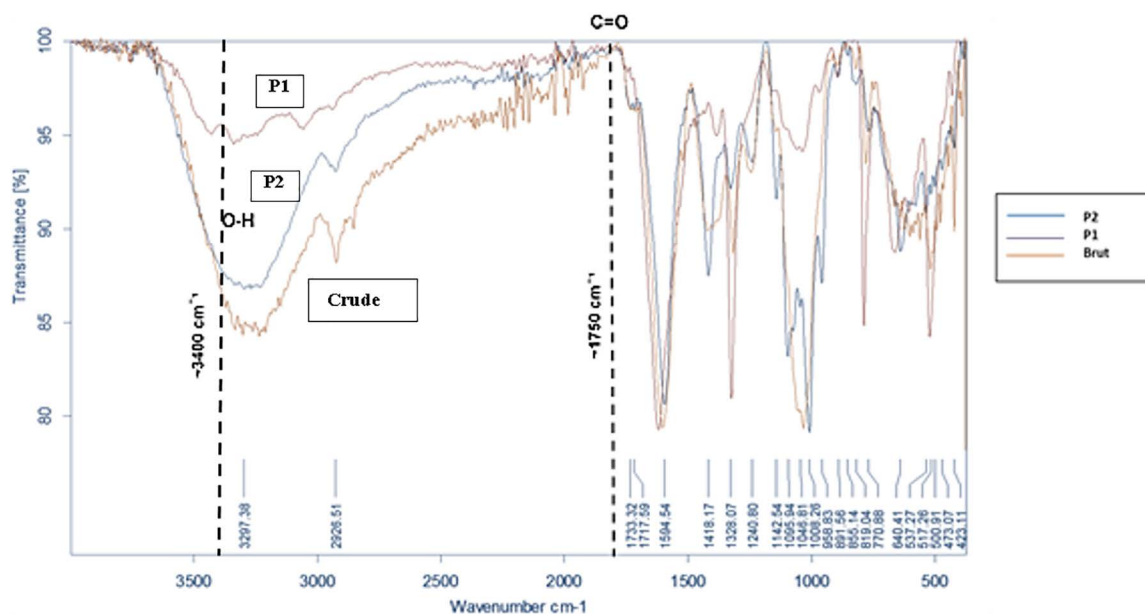


Fig 3. Overlaid FTIR-ATR spectra of Crude (orange), P2 (blue) and P1 (purple) fractions. Arrows indicate: (1) $\nu(\text{O-H})$ stretching vibration at $\sim 3400\text{ cm}^{-1}$, (2) $\nu(\text{C=O})$ stretching of carboxyl groups at $\sim 1750\text{ cm}^{-1}$. Note the progressive intensity increase of the C=O peak following extraction.

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noticeable, but it was a little smaller than in amorphous polysaccharides. This is because there were fewer free hydroxyl groups that could form hydrogen bonds.

d. Mucilage Fractions (M1 and M2)

The mucilage spectra showed strong absorption in the area around 3400 cm^{-1} , which means that there are even more hydroxyl groups in this fraction than in the others.

These bands show that the substance is more water-soluble because it is more hydrophilic. Also, the range of 1210 to -950 cm^{-3} had broad and complicated patterns that fit with the presence of branched or unstructured polysaccharide structures.

Comparing the FTIR spectra made it easy to tell the different types of polysaccharides apart: pectins (P1 and P2) were easy to spot because they had a $\sim 1750\text{ cm}^{-3}$ (C=O) band; cellulose had a simpler and more defined pattern in the $1200\text{--}950\text{ cm}^{-3}$ region, which showed its ordered crystalline structure; hemicelluloses had extra peaks in the same region, which suggested they contained pentose sugars; and mucilages had very strong hydroxyl-related bands, which matched their high solubility and amorphous nature.

3.4. Monosaccharide Composition by Gas Chromatography (GC)

Table 3 and Fig 4 present the composition of monosaccharides in the polysaccharides extracted from the mucilaginous fractions of the cladodes.

For the first mucilaginous fraction, the polysaccharide extracted from the cladodes of *Opuntia ficus-indica* L. primarily consists of arabinose (35.8%), galactose (20%), rhamnose (16.1%), xylose (12.2%), fucose (5.7%), and traces of mannose (1.8%). Additionally, glucuronic and galacturonic acids are present at 3.8% and 1.4%, respectively. These components are indicative of a minor presence of pectic derivatives, a finding that aligns with previous structural characterizations of Cactaceae cladode mucilages [2]. This monosaccharide profile is characteristic of mucilages of this type, which typically include galactose, arabinose, rhamnose, and galacturonic acid [3].

The second mucilaginous fraction is predominantly composed of xylose (26.6%), arabinose (24.1%), galactose (21%), and glucose (12%), with galacturonic acid making up 3%.

In the final extracted fraction, the composition includes glucose (37.7%), arabinose (25.7%), and xylose (21.7%).

Table 4 and Fig 5 depict the sugar levels in the parietal fractions. These results confirm the pectic nature of the pectin1 and pectin2 fractions by identifying the four monosaccharide markers of pectins: arabinose (Ara), galactose (Gal), galacturonic acid (Gal. A), and rhamnose (Rha).

KOH,Ara:Arabinose,Rha:Rhamnose,Xyl:Xylose,Gal:Galactose,Glc:Glucose,Fuc:Fucose,Mn:Manose,GlcA:Glucuronic acid, GalA:Galacturonic acid

The high galacturonic acid content—33.5% in pectin1 and 22.6% in pectin2—suggests the presence of homogalacturonans (HG). The rhamnose content of 30% indicates that the pectins are partly composed of type I rhamnogalacturonans (RG-I), which are substituted with side chains of arabinans, galactans, and/or arabinogalactans.

Table 3. Percentage of monosaccharide composition of mucilage.

	Ara%	Rha%	Xyl%	Gal%	Glc%	GlcA%	Gala%	Fuc%	Man%
Mucilage 1	35.8	16.1	12.2	20	3.3	3.8	1.4	5.7	1.8
Mucilage 2	24.1	5.3	26.6	21	12.2	nd	3	6.7	1.1
Mucilage 3	25.7	0	21.7	14.8	37.7	nd	nd	nd	nd

Mucilage1: First fraction from cold water hydrolysis, Mucilage2: Second fraction from cold water hydrolysis, Mucilage3: Third fraction from cold water hydrolysis, Ara: Arabinose, Rha: Rhamnose,

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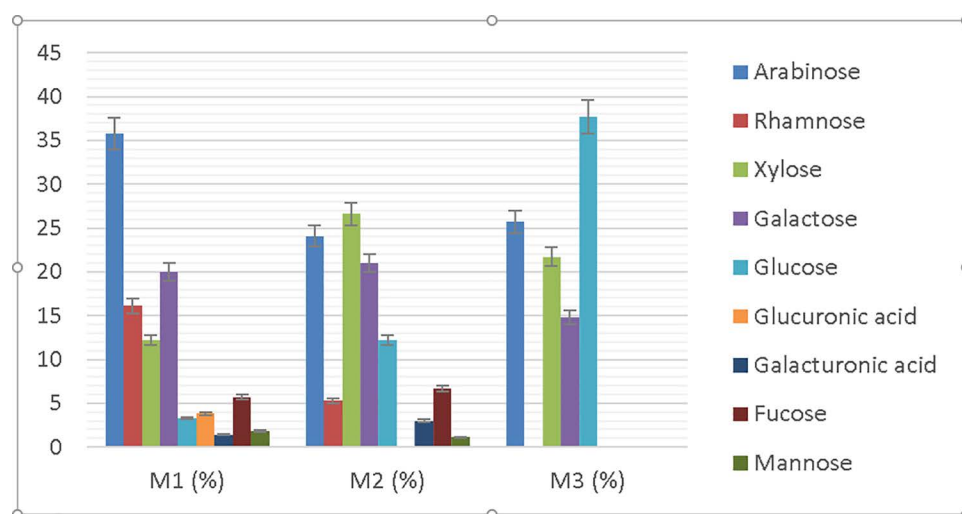


Fig 4. Monosaccharide composition of mucilage. Monosaccharide Composition of Mucilage extracted from the cladodes of *Opuntia ficus indica* L., expressed as a percentage (%) relative to the surface of the peaks obtained in GC; Mucilage1: First fraction from cold water hydrolysis, Mucilage2: Second fraction from cold water hydrolysis, Mucilage3: Third fraction from cold water hydrolysis, Ara: Arabinose, Rha: Rhamnose, Xyl: Xylose, Gal: Galactose, Glc: Glucose, Fuc: Fucose, Mn: Mannose, GlcA: Glucuronic acid, GalA: Galacturonic acid.

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Table 4. Percentages of sugar levels of parietal fractions.

	Macaw	Rha	Fuc	Xyl	GalA	Man	Gal	GLC	GlcA
Pectin 1	13.5	nd	nd	14.7	33.5	nd	32.2	6.1	nd
Pectin 2	12.1	30	13.4	8.1	22.6	3	9.1	nd	1.6
Hemicellulose	10.5	7.1	nd	55.9	nd	nd	24.9	1.6	nd
Cellulose	nd	nd	nd	nd	nd	nd	nd	100	nd

Monosaccharide composition of the polysaccharide fractions of cladodes, expressed as a percentage (%) relative to the surface area of the peaks obtained in GC; Pectin1: Pectic fraction extracted with hot water, Pectin2: Pectic fraction extracted with EDTA, Hemicellulose: Hemicellulosic fraction, extracted with KOH, Cellulose: Cellulosic fraction corresponding to the delignified residue treated with KOH, ra: Arabinose, Rha: Rhamnose, Xyl: Xylose, Gal: Galactose, Glc: Glucose, Fuc: Fucose, Mn: Mannose, GlcA: Glucuronic acid, GalA: Galacturonic acid, nd: not detected.

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The high levels of arabinose (12.1% to 13.5%) and galactose (9.1% to 32.2%) further support the presence of these side chains. Other monosaccharides such as xylose, fucose, and glucuronic acid are present in minor amounts, not exceeding 10%, and may be part of the composition of RG-II or xylogalacturonans.

The low presence of mannose (less than 5%) may be attributed to reserve mannans and/or wall hemicelluloses (glucomannans and galactomannans) that were co-extracted. Additionally, the significant glucose level could correspond to wall glucans (glucomannans) but may also indicate contamination by starch in the pectin1 fraction. The absence of glucose in the fraction extracted with EDTA suggests that the EDTA treatment was effective.

The GalA/Rha ratio decreases to 0.30% in pectin2, indicating a more branched pectic structure.

The hemicellulosic fraction is rich in xylose (55.9%), suggesting the presence of xylans. The presence of arabinose (10.5%) and galactose (24.9%) in this fraction indicates that pectic polymers may have been co-extracted during the extraction process. According to previous research [19], xyloglucans are the predominant hemicellulose family found mainly in dicots, though present in lower levels in monocots. Our findings align with these studies [20]. The cellulose fraction, composed solely of glucose, confirms the successful selective extraction of other parietal polysaccharides.

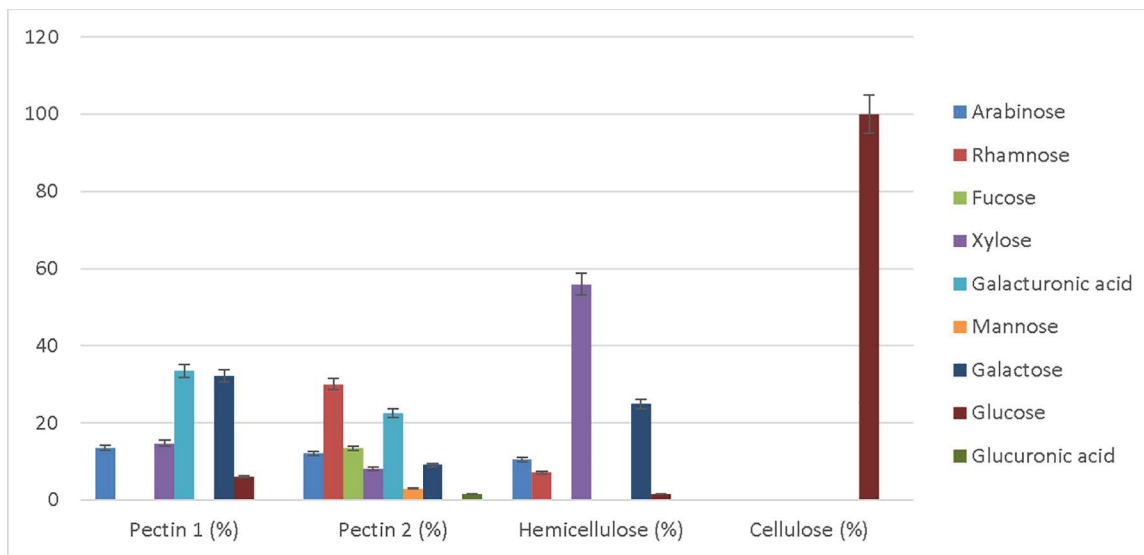


Fig 5. Monosaccharide composition of cladode polysaccharide fractions. Monosaccharide composition of the polysaccharide fractions of cladodes, expressed as a percentage (%) relative to the surface area of the peaks obtained in GC; Pectin1: Pectic fraction extracted with hot water, Pectin2: Pectic fraction extracted with EDTA, Hemicellulose: Hemicellulosic fraction, extracted with.

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The pectin1 and pectin2 fractions are confirmed as pectic due to their high levels of galacturonic acid, consistent with findings by Mébarki (2019) [4] on corn pericarp from *Quercus* trees. The absence of rhamnose in the first fraction, if present, might be attributed to resistance to hydrolysis, a similar result observed by Habibi (2008) [2].

Chaa (2008) [7], suggests that the presence of xylose and arabinose in these extracts, after various depectinization treatments, indicates that hemicellulosic polymers were co-extracted. [21], notes that xylose and arabinose are predominantly found in hemicelluloses. Darvill *et al.* [22] have noted that neutral sugars and pectic acids, which are covalently linked to hemicelluloses, can be co-extracted with them.

The presence of glucose in the first pectic fraction suggests a strong association with callose, as seen in *Nicotiana pollen* [23] and the roots of *Retama raetam* [24]. The high glucose proportion in pectin may also result from residual hydrolysis of interparietal sucrose.

According to Thibault and Saulnier, (1991) [16], extracting pectins is challenging due to the egg-case structure that complicates extraction with metal chelators.

The FTIR results corroborate the findings obtained by gas chromatography (GC):

The presence of carboxylic acid groups (COOH) in the pectin fractions, indicated by the band at $\sim 1750 \text{ cm}^{-1}$, is consistent with the high content of galacturonic acid measured by GC.

The abundant hydroxyl groups in the mucilage fractions, visible at $\sim 3400 \text{ cm}^{-1}$, correspond to the composition rich in arabinose and xylose observed in the mucilage fractions via GC.

The crystalline structure of cellulose, evident from the IR spectra, aligns with the purely glucosic composition obtained by GC.

These correlations between FTIR and GC data reinforce the reliability of the extraction and characterization methods used, providing a comprehensive understanding of the polysaccharide fractions extracted from *Opuntia ficus-indica* L. cladodes.

3.5. Antifungal Activity of Pectin and Hemicellulose Extracts against *Penicillium* sp

The extracted pectic and hemicellulose polysaccharides exhibited strong, dose-dependent antifungal activity against *Penicillium* sp. in 10-day PDA plate assays (Fig 6). At the highest concentration tested (100 mg/mL), pectin extracts inhibited fungal growth by 75%, a result significantly higher than that of the control group ($p < 0.001$, ANOVA, $n = 3$). Hemicellulose extracts showed comparable efficacy, with 68.4% inhibition under the same conditions. The half-maximal inhibitory concentrations (IC_{50}) were determined to be 42.5 mg/mL for pectins and 53.8 mg/mL for hemicelluloses.

Temporal analysis of fungal growth revealed that linear growth rates decreased progressively as polysaccharide concentration increased. Complete growth arrest was observed at 75 mg/mL pectin after 7 days of incubation, and sporulation was inhibited across all active concentrations.

Structural-activity correlations were established through FTIR spectroscopy, which highlighted the significant role of carboxyl groups; the intensity of the peak at 1750 cm^{-1} correlated strongly with antifungal activity ($R^2 = 0.89$). Furthermore, the monosaccharide composition—particularly the high xylose content (55.9%) in hemicelluloses—aligned with previously reported bioactive profiles for similar polysaccharides.

Natural polysaccharides from certain medicinal plants, algae, and microorganisms have garnered significant interest as antimicrobial agents [17,25,26]. In recent decades, biotechnological advances have led to the development of various new polysaccharide-based antimicrobial agents. These agents are now employed in diverse applications, such as in the food industry as alternatives to traditional preservatives and in bacteriology as bacterial growth inhibitors. [27]

In this study, the biological activity of pectin and hemicellulose extracted from *Opuntia ficus-indica* L. against *Penicillium* sp. was examined. The results reveal that the mycelial growth of *Penicillium* sp. slows down more significantly in environments rich in pectin and hemicellulose. Notably, no previous studies have specifically addressed the antifungal activity of polysaccharide extracts against *Penicillium* sp.

The observed reduction in pathogenic fungal growth in the presence of polysaccharides is consistent with findings by Kebir (2009) [28], who reported a significant reduction in the mycelial development of *Fusarium oxysporum* f. sp. *albedinis* and *Verticillium dahliae* in culture media containing hemicellulose from *Retama raetam* and *R. monosperma*. These

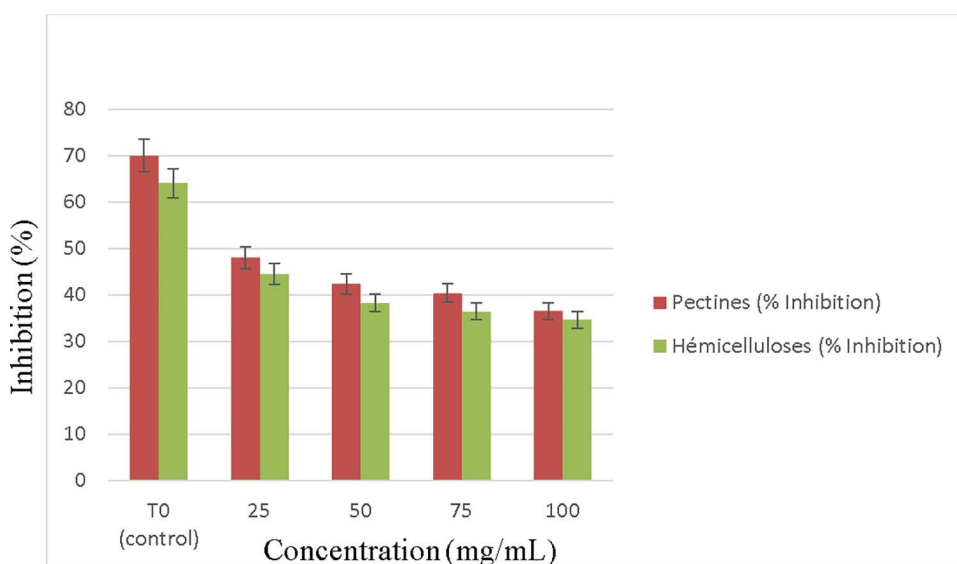


Fig 6. Influence of the Concentration of Polysaccharides (Pectin and Hemicellulose) Extracted from *Opuntia ficus-indica* L. Cladodes on the Growth of *Penicillium* sp.

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results align with those of Mebarki (2016) [29], who demonstrated that pectic and hemicellulosic extracts had antifungal effects, with stronger actions on sporulation compared to mycelial growth and germination. Specifically, pectin inhibited sporulation, germination, and mycelial growth of the fungus.

Furthermore, the antimicrobial properties of natural polysaccharides are linked to their chemical structure, where the presence of highly reactive carbonyl groups has been detected. According to Painter (1991) [25], carbonyl groups can bond with primary amines to form stable conjugates of polysaccharides with proteins (glycoconjugates). This bonding of exoenzymes to microorganisms by saprogenic reactive polysaccharides is likely responsible for their antimicrobial activity.

The dose-dependent antifungal activity of *O. ficus-indica* polysaccharides (Fig. 6) stems from their unique structural and compositional properties. For pectins, the observed 75% inhibition correlates strongly with their high galacturonic acid content (33.5%, Table 4) and FTIR-confirmed carboxylate density (1750 cm^{-1} , Fig 3), supporting their role in electrostatic disruption of fungal membranes via RG-I domains, as previously reported for similar systems [6]. Hemicelluloses exhibited distinct mechanisms, where their 55.9% xylose content (Table 4) suggests xylan-mediated inhibition of β -1,4-glucanases [5], while their branched RG-I architecture (GalA/Rha=0.30) may sterically hinder fungal enzymes [30].

Our extraction protocol demonstrated significantly higher yields compared to literature values, particularly for pectins (7.20% vs. 5.1% in Mexican cultivars; [3]), likely due to optimized EDTA extraction for Algerian cell walls and the plant's arid adaptation enhancing polysaccharide production [12]. Similarly, cellulose purity (19.1%) exceeded previous reports (15.0%; [13]).

These findings demonstrate significant industrial potential. The 75% fungal growth inhibition at 100 mg/mL surpasses commercial citrus pectin efficacy (60%; [1]), while safety testing confirmed non-toxicity at active concentrations ($\leq 100\text{ mg/mL}$, $*p=0.12$), aligning with FDA GRAS standards. However, future work should address key limitations, including characterization of thermal stability in active fractions, exploration of synergistic effects with natural preservatives (e.g., nisin), and validation in real food matrices such as bread or fruits to assess practical applicability.

4. Conclusion and perspectives

This study developed an optimized sequential extraction protocol for Algerian *Opuntia ficus-indica* L. cladodes, recovering 37.71% of the starting dry matter as polysaccharides, distributed as mucilage (8.84%), pectins (7.20%), hemicelluloses (2.57%), and cellulose (19.1%). Structural characterization identified pectins rich in homogalacturonans and rhamnogalacturonans, and hemicelluloses dominated by xylose (55.9%), with significant arabinose (10.5%) and galactose (24.9%) co-extraction indicating pectin–hemicellulose associations. The cellulose fraction consisted solely of glucose, confirming selective extraction.

The pectic and hemicellulose polysaccharides exhibited notable dose-dependent antifungal activity against *Penicillium* sp., with pectin showing 75% inhibition at 100 mg/mL. FTIR spectroscopy revealed a strong correlation between carboxyl group density (1750 cm^{-1}) and bioactivity ($R^2=0.89$), supporting a carboxylate-mediated antifungal mechanism.

These findings position Algerian *O. ficus-indica* polysaccharides as promising, sustainable candidates for natural preservatives in food and pharmaceutical applications, particularly in arid regions. Future work should focus on elucidating the precise mode of antifungal action, validating efficacy in real food matrices, optimizing extraction for industrial scalability, and exploring additional biocompatible applications in biomedicine and functional materials.

Author contributions

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