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Monosaccharide Composition And In Vitro Antioxidant, Antidiabetic, And Anticholinesterase Activities Of Pectins Extracted From Opuntia Stricta (Haw) Cladodes

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Abstract

Pectins isolated from the cladodes of Opuntia stricta (Haw.) were extracted using two methods aqueous extraction (WE) and oxalate extraction (OE) and thoroughly characterized for their monosaccharide composition as well as their bioactivities. The extractions yielded 13.84% of WE and 6.12% of OE. Gas chromatography (GC) analysis revealed a high content of neutral sugars, particularly arabinose, with highest content in OE (31.69%) and WE (24.46%).

In vitro antioxidant assays revealed that pectins obtained via oxalate extraction (OE) consistently exhibited greater activity than those obtained by water extraction (WE). OE pectins demonstrated higher DPPH radical scavenging capacity (14.63% vs. 10.21%) and metal ion chelation (33.95% vs. 27.33%). Similarly, in the β-carotene/linoleate bleaching assay, OE showed enhanced antioxidant activity (20.17%) compared to WE (12.35%). In the ABTS•+ assay at 200 µg/mL, OE pectins also outperformed WE, with scavenging activity reaching 26.54% versus 16.04%. Regarding digestive enzyme inhibition, both WE and OE pectins exhibited moderate activity (IC_{50} > 200 µg/mL), with maximum inhibitions of 23% for α-glucosidase and approximately 16% for α-amylase at 200 µg/mL. Additionally, cholinesterase inhibitory activity remained limited, with OE showing slightly higher activity than WE against butyrylcholinesterase (29% vs. 24%). These results indicate that OE pectins may possess a modestly enhanced inhibitory potential compared to WE pectins.

These results indicate that Opuntia stricta *pectins possess remarkable antioxidant activity and structural characteristics that may support neuroprotection through indirect mechanisms, despite exhibiting limited inhibition of cholinesterase and key digestive enzymes. This study enhances the understanding of* O. stricta *pectins functional properties and underscores their potential as bioactive ingredients in nutraceutical and pharmaceutical applications.*

Keywords: *Opuntia stricta (Haw), Pectins, Biological Activities, GCMS.*

INTRODUCTION

The Cactaceae family includes the Opuntia genus, which is one of the most diverse. It is mainly native to the Americas. These succulent plants have developed remarkable adaptive strategies to survive in extreme environments. These environments are subject to drought, intense heat and often poor or saline soils. Their morphology (cladodes, mucilage, water

The genus Opuntia is one of the largest and most diverse groups in the Cactaceae family, of which it constitutes a major clade. The geographical origin of this species is the American continent. It is characterised by its optimal adaptation to environments with low rainfall, whether arid or semi-arid. This ability is the result of highly specialised morphological and physiological mechanisms. Mexico is recognised as the main centre of origin, diversification and domestication of the Opuntia genus. It contains the greatest variety of species in the world, with wild, semi-domesticated and cultivated forms used for food, medicine and fodder. According to Li et al., (2025), recent phylogenetic analyses confirm that there are approximately 1,500 species divided into several subgenera in the subfamily Opuntioideae and the tribe Opuntieae. Among the many plant species studied, Opuntia ficus-indica (L.) Mill. stands out for its scientific importance and commercial exploitation. This plant is of major economic importance, particularly thanks to its edible fruits, known as 'prickly pears', which are rich in bioactive pigments, organic acids and vitamins. The young cladodes of this plant are also consumed as vegetables, further enhancing its nutritional value and impact on human nutrition. As demonstrated by Liu et al., (2025), fruits and vegetables are currently valued for their high fibre, pectin and polysaccharide content.

However, some species of the genus are known for their invasive behaviour, particularly O. stricta (Haw.), O. ficus-indica and O. monacantha. The study conducted by Misuri et al., (2025) demonstrated that, once established, O. stricta alters the composition of plant communities and reduces species richness in Mediterranean ecosystems, thereby affecting agricultural productivity and natural regeneration.
According to observations by Novoa et al., (2021), who showed that Opuntia stricta modifies soil structure by creating 'islands of fertility,' which increases nutrient availability and promotes its own expansion at the expense of native species. From a pharmacological point of view, Opuntia species play a major role in traditional medicine and ethnopharmacology. Recent studies have highlighted their use in the treatment of chronic metabolic diseases. These include type 2 diabetes, obesity and high blood pressure. They are also used in the prevention of certain cancers. Studies conducted by Chen et al., (2023) have demonstrated the therapeutic applications of cactus polysaccharides, which have antioxidant, anti-inflammatory and immunomodulatory effects. He et al., (2023) reported that specific purified pectins isolated from Opuntia ficus-indica exhibit strong inhibitory activity against the digestive enzymes α-amylase and α-glucosidase, highlighting their promising antidiabetic potential. Owing to their high molecular weight and complex structural features, these biopolymers display both antioxidant and therapeutic properties, supporting their potential application in the formulation of functional foods and natural nutraceuticals (Liu et al., 2025).
Although Opuntia ficus-indica has been extensively investigated, Opuntia stricta remains comparatively underexplored, particularly with respect to the chemical composition and bioactive potential of its pectins. Notably, the structural features of polysaccharides — which play a pivotal role in determining their biological functionality — can vary substantially among species, plant tissues, and extraction methodologies.

MATERIAL AND METHODS

Extraction of pectins

Opuntia stricta Haw. cladodes growing in the West of Algeria, were collected in an open field on the edge of the University of Sciences and Technology, Mohamed Boudiaf, Oran in 2022.
To prepare the cell wall residue, twenty grams of milled powder were successively extracted twice with a 50/50 ethanol-chloroform solution at room temperature for 14 hours. The supernatant was then discarded to remove soluble lipids and pigments. To remove any traces of chloroform, the residue was filtered through blotting tissue and mixed with 80% ethanol under constant stirring for two hours. After three cycles of washing with distilled water and acetone solution, the residue was dried at 60°C in a ventilated oven and then weighed.
For pectic polysaccharides extraction, dried defatted residue was firstly refluxed with boiling water for 2x 2 h, 100°C to obtain Water Fraction Pectins (WF). The residual residue was processed for 4 h at 80°C with a 1% ammonium oxalate solution. To obtain Oxalate Fraction Pectins (OF), the residual residue was treated with 1% ammonium oxalate solution at 80°C for 6 h. The fractions were separated from the supernatant by filtration through sintered glass (porosity 3). Dialysis was then carried out using a Spectrapor membrane with a cutting threshold between 6,000 and 8,000 Da. The crude pectins fractions were obtained after centrifugation (12,000 rpm), filtration, and lyophilization.

Colorimetric assay of total sugars

The uronic acid content of the pectin fractions was determined using the meta hydroxydiphenyl method (Blumenkrantz & Asboe-Hansen, 1973) with galacturonic acid as standards. Total sugar content of pectin fractions was measured by phenol-sulfuric acid colorimetric methos (Dubois et al., 1956), using glucose as the standards.

Analysis of monosaccharide composition

The monosaccharide composition of the extracted pectins was determined using gas chromatography coupled with mass spectrometry (GC-MS) on a Shimadzu device (Varian Saturn 2100T, USA). This method was adapted from Zhang et al., (2016), with slight modifications. The pectin fractions were subjected to methanolysis before analysis.

Preparation Of Trimethylated Methyl Glycosides Derivatives

For the preparation of trimethylated derivatives of methylglycosides, the analysis was performed on 200 to 500 µg of freeze-dried pectin powder, added with an internal standard, mesoinositol (MI), at a concentration of 10% of the polysaccharide quantity (Marga et al., 1995).

Monosaccharides were recovered as methylglycosides by mixing 1 mL of 1 M hydrochloric methanol with the anhydrous pectin sample. The methanolysis was stopped by evaporation of the hydrolyzate under a nitrogen flux after 24 hours at 80°C in a sealed tube.
The residue was dissolved in 1 mL methanol, then delipidated by 3 x 1 mL heptane extractions. It was evaporated again under nitrogen flux.
Afterwards, the methylglycosides were trimethylated using 100 µL of N,O-bis-trim ethylsilyl-trifluoroacetamide (BSTFA) and 100 µL of pyridine containing 1% trimethylchlorosilane (TMCS) for two hours at 27°C in the dark.
The standard sugars (Ara, Rha, Fuc, Xyl, Man, Gal, Glc, and Mal) were converted into TMS derivatives using the same process. Once cooled, sample derivatives were injected into GC-MS (Varian Saturn 2100T, USA) fitted with a 30 m × 0.32 mm × 0.25 mm HP-5 fused silica capillary column.
The carrier gas is Helium (He) with a flow rate of 1 mL/min. The injector and detector temperatures were fixed at 250 °C and 270 °C, respectively. The temperature of the column was fixed at 100°C for 5 minutes, rose to 150°C at a rate of 5°C/min and held there for 5 minutes, increased to 240°C at 5°C/min and finally kept at 240°C for 2 min.
Monosaccharides were identified by comparison of their retention times with authentic standards and the results were expressed as a molar percentage calculated by the area normalization method .

Determination of antioxidant activity

A variety of tests were used to monitor the antioxidant activities of polysaccharide fractions, including metal-chelating activity tests on Fe^{2+}, ABTS cation-trapping tests, and DPPH free radical scavenging tests and the β-carotene-linoleic acid test (Talal Ahmedah,2023). EDTA, α-tocopherol and BHA were used as antioxidant standards. Eight concentrations (6.25, 12.5, 25, 50, 100, 200, 400, and 800 µg/ml) of polysaccharide fractions were used for assessing antioxidant activity. The concentration of the sample exhibiting 50% inhibition (IC50 µg/ml) was determined from the graph plotting percentages of antioxidant activity (inhibition %) in comparison to sample concentrations (µg/ml). The results of antioxidant activity were expressed as 50% inhibitory concentration (IC50) and A0.50, corresponding to the concentration providing an absorbance of 0.500 for the copper reducing antioxidant capacity (CUPRAC) test and the percentage inhibition (%) at a concentration of 200 µg/ml in the metal chelation test (**Tel** et al**., 2012.).**

Anticholinesterase activity

This study measured the inhibitory activity of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) using the spectrophotometric method developed by Ellman et al., (1961). Briefly, 10 µL of sample solution and 20 µL AChE (or BChE) were added to 150 µL of 100 mM sodium phosphate buffer (pH 8.0). The solutions were shaken and incubated at 25 °C for 15 minutes, then 10 µL of DTNB was added to the mixture. The reaction was started by the addition of 20 µL acetylthiocholine iodide (or butyrylthiocholine iodide). Microplate reader at 412 nm was used to monitor the hydrolysis of these substrates. Galantamine was used as the reference compound. The results were expressed as the percentage inhibition (%) of the enzyme at a concentration of 200 µg/ml of pectins fraction.

Antidiabetic activity determination

Determination of α-amylase inhibitory activity

The α-Amylase inhibitory activity of pectin fractions was determined using a slightly modified method (Quan et al., 2019 and et al., 2022). 50 µL of α-amylase (0.1 units/ml) in phosphate buffer (20 mM pH = 6.9 prepared with 6 mM NaCl) and 25 µL of sample solutions were mixed in microplate and pre-incubated at 37 °C for 10 min. Then 50 µL starch solution (0.05%) was added and incubated at 37 °C for 10 minutes. The reaction was completed by addition of HCl (0.1 M, 25 µL) and Lugol (100 µL) solutions. Absorbance was measured at 565 nm and acarbose was used as standard.

Determination of α-glucosidase inhibitory activity

The Procedure was adapted from Kim et al., (2000) with slight modification, utilizing acarbose as a positive control. A total of 50 µL phosphate buffer (0.01 M, pH 6.9,) 25 µL p-nitrophenyl-α-d-glucopyranoside (p-NPG) 50 mM (dissolved in 0.1 M buffer phosphate pH 6.9), and 10 µL of sample solution were incubated for 20 min at 37 °C. After incubation, 25 µL of α-glucosidase 0.1 Unit/mL in 0.1 M phosphate buffer pH 7.0 was added and then incubated at 37 °C for 30 min. The reaction was stopped by adding 100 µL of Na2CO3 (0.2 M) and then measured at 400 nm.

IC50 and A0.50 values

The results are presented in terms of the 50% inhibition concentration (IC50) in ABTS+, β-carotene-linoleic acid, DPPH, Metal chelating assay, AChE, BChE, α -amylase, and α -glucosidase enzyme inhibition assays.

The results of CUPRAC assays were given as A0.50, which corresponds to the concentration producing 0.500 absorbances comparing that those of standards.

Statistical analysis

All experiments were conducted in triplicate and the data was presented as the mean value ± standard deviation (SD). The statistical significance was evaluated by t-test (SPSS v. 22.0 program).

RESULTS AND DISCUSSION:

Yield of extraction of pectin fractions

The pre-treatment with ethanol-chloroform solution was used to remove liposoluble components such as pigments and waxes. This treatment yielded an insoluble cell wall residue (37.78 % of the cladode of Opuntia… powder). The pectin fractions were obtained from water extract (WE) and oxalate extract (OE) after ethanol precipitation.

yield and the uronic acid content of the pectin fractions obtained are reported in Table 1.

Table 01: Extraction yields and uronic acid content of pectins extracted from Opuntia stricta (Haw) cladodes.

	Yield (%)	**Uronic acid** (%)
Cell wall residue	37.78	-
WE	13.84	37.8
OE	6.12	35.9

WE, water extract; OE, oxalate extract;

The total yield of WE and OE were 13.87% (13.87g WE from 100 g dried cell wall residue) and 6.12% (6.12 g OW from 100 g dried cell wall residue), respectively. The results indicate that the yield of pectins solubilised in water is twice as high as that of pectins solubilised in oxalate. Compared to previous studies, the yield obtained from the cladode WE was relatively similar to those reported in Opuntia ficus indica (12.4 %) and (16.3 %) (Habibi et al. 2004; and Kalegowda et al. 2017), respectively, but higher than that obtained under the microwave extraction process (7%) (Majdoub et al., 2010).

The difference in yield results suggests that the amount of yield obtained from the extraction process is determined by the extraction method, and type of solvent (Dardavilla et al., 2023).

These results revealed that the pectin fractions had a similar uronic acid (37.8% and 35.9%, respectively). This hign content was confirmed their pectic nature.

In cacassae, several parameters can influence the composition of pectins in uronic acid and neutral oses, such as the organ analyzed, the fraction studied and even the extraction method. (Liu et al ., 2025).

Monosaccharide composition

The monosaccharide compositions of the pectin extracts were analysed using GC-MS.

The results are given in Table 2.

Table 2: Monosaccharide composition of the pectin fraction extracted by sing hot water and oxalate ammonium from Opuntia strica (Haw) cladods.

Fraction		**Monosaccharides (% molar)**							
	Ara	**Rib**	**Man**	**Xyl**	**Gal**	**Glc**	**GlcA**	**GalA**	
WE	24.16	22.89	16.92	3.8	8.66	18.86	0.72	1.45	
OE	31.68	22.73	17.08	2.99	7.96	15.91	-	0.68	

WE, water extract; OE, oxalate extract

Results of pectin fractions extracted with water (WE) and amonum oxalate (OE) and characterised by GCMS revealed that the Ara wase detected in highest monosaccharide content in both WE (24.46%) and OW (31.69%).

This monosaccharide is often found in the side chains of the branched regions of RGI pectins, typically in the form of arabinogalactans and arabinans linked to the rhamnose-rich backbone. The high arabinose content detected in the OE fraction may be attributed to the oxalate extraction method, which effectively releases more of the highly branched side chains from the plant cell wall.

It is worth noting that the presence of ribose at a concentration of approximately 23% in both fractions is atypical when compared to reference pectins. According to Mohnen (2008), the detection of ribose may result from contamination with other residues, such as ribosylated polysaccharides specific to certain Cactaceae species. Additionally, Medina-Torres et al. (2013) suggest that the presence of ribose could be associated with specific metabolic adaptations in desert plants, involving ribosylated glycosides that play a role in water retention or defense mechanisms.

Additionally, mannose is found in comparable proportions in both fractions (16.92% and 17.08%), despite not being a major component of homogalacturonans. Its presence may originate from heteromannans or glucomannans associated with the plant cell wall, which are often co-extracted alongside pectins (Voragen et al., 2009). Furthermore, the analysis revealed that xylose content remained below 4%, indicating a limited presence of xylogalacturonans (XGAs). These are substituted derivatives of the homogalacturonan (HG) backbone, in which one or more xylose residues (β-D-Xylp) are attached to the O-3 (and occasionally O-4) position of galacturonic acid (GalA) units (Harholt et al., 2010; Mohnen, 2008).

This low content indicates that XGA is not a major structural feature of these pectins. Cell wall studies have shown that homogalacturonan is the major pectin (60-65%) while XGA and rhamnogalacturonan II (RG-II) are minor components (<10%) (Harholt et al., 2010; Ralet et al., 2003). Galactose (8%) is a marker for galactan side chains, which are typical for RG-I regions (Mohnen, 2008). These residues are involved in cell wall flexibility and hydration and are in agreement with the high arabinose content.

The galacturonic acid contents 1.45% in the WE fraction and 0.68% in the OE fraction were lower than those typically found in pectins from conventional sources, where homogalacturonans (HG) generally account for 60–70% of the total composition (Willats, Knox & Mikkelsen, 2006). This low galacturonic acid content suggests that the examined pectins possess a highly branched structure, dominated by rhamnogalacturonan I (RG-I), and are largely devoid of continuous homogalacturonan regions a feature previously observed in pectins extracted from secondary plant tissues) (Voragen et al., 2009; Mohnen, 2008**.** The high glucose content (15–19%) may be attributed to contamination with co-extracted cellulose or hemicellulose residues, as reported by Voragen et al. (2009).

The high proportion of neutral sugars may reflect the complexity of the cell wall matrix in the Opuntia stricta (Haw.) cladodes. Glucuronic acid was detected in trace amounts, below 1%. Although uncommon in pectins, glucuronic acid has been identified in specific structural domains such as RG-II and in minor uronic acid-containing polysaccharides (O'Neill, Ishii, Albersheim, & Darvill, 2004). Overall, the pectins extracted from Opuntia stricta (Haw.) appear to be predominantly composed of highly branched rhamnogalacturonan I (RG-I), rich in arabinan and arabinogalactan side chains, with a limited homogalacturonan (HG) content. The presence of non-pectic polysaccharides suggests possible co-extraction from the cell wall matrix.

This profile, marked by a predominance of neutral sugars, is consistent with findings reported for other species of the Opuntia genus (Goycoolea & Cárdenas, 2003; Habibi et al., 2004). According to these studies, pectins from certain Cactaceae, particularly those with an RG-I backbone, can be strongly masked by long neutral side chains such as arabinan and arabinogalactan. This structural feature often results in a low relative proportion of rhamnose among total monosaccharides. When the side chains are particularly large, the molar contribution of rhamnose becomes almost negligible in the overall monosaccharide composition.

Antioxidant activity

The results presented in the table 03 show the antioxidant activities of polysaccharides extracted from the cladods of Opuntia stricta (Haw) , evaluated using β-carotene bleaching, DPPH• and ABTS•+ radical scavenging, CUPRAC and iron chelation tests. The results are expressed as a percentage of inhibition (%)

at a concentration of 200 µg/mL and as the 50% inhibitory concentration (IC50), as well as A 0.50 values, which correspond to the concentration producing an absorbance of 0.500 for the copper reduction antioxidant capacity (CUPRAC) test.

Table 03. Antioxidant activity of the samples by β-Carotene-linoleic acid, DPPH•, ABTS•+, CUPRAC and metal chelating assays a of pectins from Opuntia strica (Haw) cladods .

Samples/ Standards	β-Carotene-linoleic acid assay		DPPH• assay		ABTS•+ assay		CUPRAC assay		Metal chelating assay	
	IC_{50} (µg/mL)	Inhibition (%) (at 200 µg/mL)	IC_{50} (µg/mL)	Inhibition (%) (at 200 µg/mL)	IC_{50} (µg/mL)	Inhibition (%) (at 200 µg/mL)	$A_{0.50}$ (µg/mL)	Absorbance (at 200 µg/mL)	IC_{50} (µg/mL)	Inhibition (%) (at 200 µg/mL)
WE	>200	12.35± 0.47	>200	10.21± 0.17	>200	16.04± 0.45	>200	0.12±0. 03	>200	27.33± 0.51
OE	>200	20.17± 0.63	>200	14.63± 0.25	>200	26.54± 0.72	>200	0.18±0. 01	>200	33.95± 1.02
α-Tocopherol	2.10±0 .05	95.73± 0.44	38.20± 0.50	84.25± 0.36	34.75± 0.55	83.63± 0.34	60.45± 0.30	70.12±0 .15	NT	NT
BHA	1.50±0 .03	96.20± 0.28	19.50± 0.30	87.90± 0.44	12.70± 0.10	88.93± 0.21	25.40± 0.42	85.67±0 .36	NT	NT
EDTA	NT	NT	NT	NT	NT	NT	NT	NT	5.50±0 .25	94.40± 0.35

[a] Values represent the means ± SEM of three parallel sample measurements ($p < 0.05$).
NT: not tested. WE: Water Extract Pectins; OE: Oxalate Extract Pectins.

The results of our study indicate that pectins extracted with oxalate (OE) exhibit slightly higher antioxidant activity compared to those extracted with water (WE). As shown by the assay data, DPPH• radical scavenging activity was 14.63% for the OE fraction and 10.21% for the WE fraction. Similarly, in the metal chelation assay, inhibition reached 33.95% for OE and 27.33% for WE, further supporting the enhanced antioxidant potential of the oxalate-extracted pectins.

For the β-carotene/linoleate assay, WE pectins exhibited modest antioxidant activity, with an inhibition rate of 12.35 ± 0.47%. In contrast, OE pectins showed higher inhibition at 20.17 ± 0.63% when tested at a concentration of 200 µg/mL. Similarly, in the ABTS•+ assay, OE pectins showed greater antioxidant potential (26.54 ± 0.72% inhibition) compared to WE pectins (16.04 ± 0.45%). These findings suggest that the oxalate extraction method favors the recovery of compounds with enhanced antioxidant activity. The CUPRAC assay, which measures the reducing capacity via cupric ion reduction, yielded relatively low absorbance values for both extracts 0.12 ± 0.03 for WE and 0.18 ± 0.01 for OE indicating a limited reducing power of the extracted polysaccharides compared to standard antioxidants.

In our study, α-tocopherol, BHA, and EDTA showed strong antioxidant activity, with IC_{50} values between 1.50 and 38.20 µg/mL and inhibition rates above 85%. EDTA was particularly effective in the metal chelation assay (94.40% inhibition, IC_{50} = 5.50 µg/mL). In comparison, Opuntia stricta pectins showed limited antioxidant capacity, especially in DPPH• and ABTS•+ assays. Oxalate-extracted pectins (OE) performed better than aqueous extracts (WE), likely due to structural differences. These results align with previous findings highlighting the role of pectin structure in determining antioxidant potential (Chandel et al., 2022; Yan et al., 2023).

Water-soluble polysaccharides extracted from Opuntia stricta fruit peel showed moderate antioxidant activity, with an IC_{50} of 6.5 mg/mL (Koubaa, 2015). The antioxidant potential of pectins is strongly influenced by their botanical origin and extraction method. In a related study, Li et al. (2016) reported 35–50% DPPH radical inhibition for Opuntia dillenii polysaccharides, depending on the concentration.

According to Li et al. (2020), the antioxidant activity of isolated polysaccharides, evaluated by the DPPH test, exhibited moderate radical scavenging capacity, considerably lower than that of ascorbic acid. This supports the conclusion that isolated polysaccharides have a limited antioxidant effect compared to total extracts. Similar results were reported by Bougandoura (2017), confirming the reduced antioxidant activity of isolated polysaccharides relative to whole extracts.

In a detailed investigation of the antioxidant properties of pectin extracted from seeds, Opuntia ficus-indica seed oil exhibited an IC_{50} of 19.79 µL/mL, indicating significant antioxidant activity (Ghazi et al., 2015). Another study on pectin extracted from the fruit skin of Opuntia ficus-indica reported an IC_{50} value of 12.99 mg/mL, demonstrating strong antioxidant capacity (Amrane-Abider et al., 2023). The variability in pectin's biological activity is attributed to differences in galacturonic acid content, degree of methylation and branching, as well as the presence of specific substructures like rhamnogalacturonan-I (RG-I) and rhamnogalacturonan-II (RG-II). These structural features directly influence their antioxidant properties, as confirmed by several previous studies (Li et al., 2025; Beukema et al., 2020; Yan et al., 2025).

Anticholinesterase activity

Alzheimer's disease (AD) affects over 20 million people worldwide and is the most common neurodegenerative disorder. Inhibiting AChE and BChE enzymes is a key approach for its treatment (Perry et al., 2008; Tel-Çayan et al., 2020). Due to limited effectiveness and side effects of current drugs, research is focused on discovering new plant-based therapies to reduce neuronal loss and aid brain recovery (Collins et al., 2011; Esposito and Cuzzocrea, 2010).

Table 04. Anticholinesterase inhibitory activities of pectins extracts from cladodds of opuntia stricta (Haw).

	Cholinesterase inhibitory activity			
Samples/ Standards	**AChE**		**BChE**	
	IC_{50} (µg/mL)	**Inhibition (%) (at 200 µg/mL)**	**IC_{50} (µg/mL)**	**Inhibition (%) (at 200 µg/mL)**
WE	**>200**	**15.60±0.51**	**>200**	**24.05±0.78**
OE	**>200**	**16.94±0.42**	**>200**	**29.18±0.75**
Galantamine	**5.50±0.20**	**89.25±0.48**	**42.20±0.35**	**79.43±0.60**

[a] Values represent the means ± SEM of three parallel sample measurements ($p < 0.05$).
WE: Water Extract Pectins; OE: Oxalate Extract Pectins.

In our study, pectins extracted from Opuntia stricta (Haw) showed low to moderate cholinesterase inhibition, with no IC_{50} reached at 200 µg/mL. Furthermore, the maximum inhibition at this concentration ranged from 15–17% for AChE and 24–29% for BChE, with slightly higher activity observed for oxalate-extracted pectins (OE), particularly against BChE. In contrast, the reference compound galantamine exhibited strong inhibition, with an IC_{50} of 5.50 ± 0.20 µg/mL for AChE (≈89%) and 42.20 ± 0.35 µg/mL for BChE (≈79%).

This difference underscores the strong pharmacological effect of galantamine and suggests that isolated pectins lack direct inhibitory activity against AChE and BChE. Their neuroprotective effect is likely mediated through other mechanisms, such as antioxidant, anti-inflammatory, or immunomodulatory actions (Li et al., 2016; Marucci et al., 2021). Previous studies on other Opuntia parts (Amrane-Abider et al., 2022; Jabir et al., 2018) reported higher cholinesterase inhibition, mainly attributed to flavonoids and carotenoids rather than polysaccharides. Therefore, O. stricta pectins may serve as complementary neuroprotective agents but cannot substitute established cholinesterase inhibitors like galantamine.

Table 05 Antidiabetic activities of pectins extracts from cladodds of opuntia stricta (Haw). Water Extract Pectins, WE pectins ; oxalate extract Pectins, OE pectins

	Anti-diabetic activity

Samples/ Standards	α-glucosidase		α-amylase	
	IC_{50} (µg/mL)	Inhibition (%) (at 200 µg/mL)	IC_{50} (µg/mL)	Inhibition (%) (at 200 µg/mL)
WE pectins	>200	19.07±0.15	>200	13.06±0.27
OE pectins	>200	23.19±0.55	>200	15.81±0.22
Acarbose	128.5±0.62	56.70±0.75	32.50±0.45	82.10±0.27

[a] Values represent the means ± SEM of three parallel sample measurements ($p < 0.05$).

Antidiabetic activities

α-Amylase and α-glucosidase inhibitors help reduce glucose release and delay carbohydrate absorption in the human body (Wang et al., 2018). Pectins extracted from Opuntia stricta cladodes showed moderate inhibitory activity against these digestive enzymes. Both aqueous (WE) and oxalate extracted (OE) pectins had IC_{50} values > 200 µg/mL, with inhibition rates at 200 µg/mL of 19.07 ± 0.15% for α-glucosidase and 13.06 ± 0.27% for α-amylase, indicating limited efficacy.

Furthermore, statistical analysis revealed a significant difference between WE and OE pectins, with inhibition rates of 23.19 ± 0.55% and 15.81 ± 0.22%, respectively, suggesting a greater affinity of WE for α-glucosidase and a lower sensitivity of α-amylase to OE. In contrast, the reference inhibitor acarbose showed much higher activity (IC_{50} = 128.5 ± 0.62 µg/mL for α-glucosidase and 32.5 ± 0.45 µg/mL for α-amylase), with inhibition rates of 56.70 ± 0.75% and 82.10 ± 0.27% at 200 µg/mL. These findings suggest that O. stricta pectins act more as dietary fibers, reducing sugar absorption through physical mechanisms rather than direct enzymatic inhibition. By comparison, O. ficus-indica whole fruit extract exhibited stronger activity after simulated digestion, with 63.4 ± 1.53% inhibition of α-amylase at 25 mg/mL and 46.5 ± 1.45% inhibition of α-glucosidase at 30 mg/mL (Medina-Pérez et al., 2019).

According to Abdel-Hameed et al. (2014), polysaccharide extracts from Opuntia dillenii cladodes and flowers exhibit moderate inhibition of α-glucosidase (25–35%) and α-amylase (20–30%) at 200 µg/mL. Kalungia et al. (2018) confirmed the antidiabetic potential of O. stricta extract in alloxan-induced diabetic mice, with significant reductions in blood glucose. Similarly, Hwang et al. (2016) reported α-glucosidase inhibition in rats treated with Opuntia extract. However, as Prashar et al., (2018) noted, O. stricta's hypoglycaemic effects are not solely due to enzyme inhibition but also involve mechanisms such as islet regeneration, insulin-like activity, and oxidative stress modulation. According to Gouws et al., (2019), the antidiabetic properties of O. dillenii and O. ficus-indica cladodes are largely attributed to their high fiber and mucilage content, which slows glucose absorption and improves metabolic regulation. Thus, the modest in vitro enzyme inhibition observed for O. stricta does not exclude a broader in vivo antidiabetic effect, likely mediated through complementary physiological pathways.

CONCLUSION

This study provides valuable insight into the structural complexity and bioactive potential of pectins extracted from Opuntia stricta (Haw.) cladodes using aqueous and ammonium oxalate methods. The predominance of neutral sugars in their monosaccharide composition reflects a highly branched polysaccharide structure. Biological evaluations revealed that oxalate-extracted pectins (OE) consistently exhibited greater antioxidant activity than water-extracted pectins (WE). Overall, these findings suggest that O. stricta pectins may exert neuroprotective and antidiabetic effects primarily through indirect mechanisms, such as the modulation of oxidative stress or interference with nutrient absorption. The combined antioxidant and structural attributes highlight their promise as complementary bioactive agents, particularly for incorporation into nutraceutical formulations aimed at managing oxidative and metabolic disorders.

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