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# Physicochemical and functional characterization of dehydrated cladodes from *Cylindropuntia imbricata*, a wild cactus

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**Abstract.** *Cylindropuntia imbricata*, belonging to the cactus family, is not edible but exhibits similar characteristics to *Opuntia* spp. (prickly pear). The information about *C. imbricata* is scarce, prompting this study to investigate the physicochemical and functional attributes of flour derived from cladodes aged 3 to 4 months. The analysis revealed that its proximate composition—moisture, ash, fats, total sugars, and fiber—mirrors that reported for various *Opuntia* spp. EDX analysis showed that $Ca^{2+}$ and $K^{+}$ are the most abundant ions, alongside Cl, P, Mg, S, and Al. The flour is notably rich in antioxidants, containing polyphenols (1308.7±1.25 mg/100 g DW), flavonoids (232.68±2.18 mg/100 g DW), flavonols (84.20±0.15 mg/100 g DW), proanthocyanidins (29.52±0.05 mg/100 g DW), vitamin E (112.23±0.92 mg/100 g DW), tannins (133.20±0.31 mg/100 g DW), and chlorophylls (23.95±0.17 mg/100 g DW). A notable functional trait is its high-water retention capacity (12.94±0.03 g $H_2O$/g flour), which could significantly improve the texture of food products. These findings indicate that *C. imbricata* flour could serve as a functional ingredient in various foods or as a source of bioactive molecules for the pharmaceutical or food industries.

***Keywords:*** *Cardenche, C. imbricate, bioactive compounds, functional properties, nopal.*

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## Introduction

Cacti are plants that grow in desert and semi-desert climates, comprising around 125 genera and over 2,000 species, with 16 genera found in North America (Elpel´s, 2018). In Mexico, 675 species belonging to the Cactaceae family are recognized (Gámez *et al*., 2016). The genus *Cylindropuntia* is one of the most representative, with about 30 species documented in Mexico (Hunt, 2016). This species was described by Haw F.M. Knuth and published in Kactus ABC 125 in 1935. The species is native from North America, it is distributed in the central northern part, the Altiplano, and the Bajío of Mexico. *Cylindropuntia imbricata* is commonly known as cardenche, cardon, or “entraña”, growing as a branched and spiny shrub 1 to 3 meters tall. Its stems are cylindrical, formed by segments 8 to 25 cm long and 1.5 to 4 cm in diameter (Hernández *et al*., 2007). This plant is considered an opportunistic and invasive as it tends to displace native vegetation (Deltoro Torró *et al*., 2014). The plant's main use is ornamental, with dehydrated cladodes used for crafts, and glochids and spines used ceremonially. The gum has been used to produce chewing gum, and during droughts, the fruits are used as fodder (Anderson *et al*., 2001). As it belongs to the Cactaceae family like prickly pear, *C. imbricata* is thought to have similar physicochemical and functional properties, though these have been little studied. Research on aqueous and

ethanolic extracts from *C. imbricata* and *C. leptocaulis* shoots found that they stimulate mycelial growth of *Fusarium* sp. and *Aspergillus* sp. fungi, contrary to the intended effect (Galán *et al.,* 2021). Another study characterized the fruit of *C. imbricata*, known as "xoconostle," finding it to be an important source of fiber, proteins, minerals, and natural antioxidants (Laguna *et al*., 2022). The presence of polyphenols in the seeds has also been reported (Reyes-Corral *et al*., 2022). The information on the bioactive compounds, chemical composition, or antioxidant activity of the cladodes is scarce. Therefore, this study aimed to perform a physicochemical and functional characterization of dehydrated cladodes of *C. imbricata* collected in Zacatecas, Mexico. The generated information contributes to the knowledge of the plant's potential for use in the food, medical, and pharmaceutical industries.

## Material and Methods

### *Plant material*

Cladodes of *C. imbricata*, aged 2 to 3 months, were collected from a location in Zacatecas, Mexico (22° 13' 35.76" N and 101° 44' 19.85" W). After removing the spines, they were washed with water to eliminate impurities. The cladodes were then placed on absorbent paper to remove excess water. The clean cladodes were sliced to a thickness of 0.3 cm and dehydrated in a convection oven (LabTech, model LDO-080F, Mexico) at 50 °C until reaching a constant weight (approximately 24 h). The dehydrated cladodes were ground using a coffee grinder (Hamilton Beach, model 80385, Mexico). The resulting powder was sieved through a 50-mesh screen to obtain a particle size of 300 µm and stored in plastic containers at room temperature for subsequent analysis.

### *Proximate composition*

Moisture content was determined by weight loss after drying the sample in an oven (LabTech®, model LDO-080F, Mexico) at 105 °C for 4 h. The ash content was determined by calcination of the sample in a muffle furnace (Felisa®, Model FE-361, Mexico) at 550 °C until white ash was obtained. Total fat was determined by gravimetric method using a Soxhlet apparatus with ethyl ether for extraction. To determine total dietary fiber, the TDF kit (Sigma®, Aldrich, St. Louis Missouri, USA) was used according to the supplier's instructions. For total carbohydrate determination, the sample was prepared according to Monrroy *et al.* (2017), and quantification was performed using the anthrone-sulfuric acid method, with glucose as the standard and absorbance read at 625 nm (Tecan® spectrophotometer, model Infinite Pro-200, Austria). Proteins were determined by the Kjeldahl method (Labconco®, Model RapidStill, Kansas City, MO. USA). All assays were performed in triplicate.

### *Mineral composition*

The mineral composition was determined by elemental mapping using Energy Dispersive X-ray Spectroscopy (EDX) with a scanning electron microscope (model JEOL JSM-7600F, Japan).

### *Bioactive compounds*

The ethanolic and methanolic extracts were prepared by mixing 5 g of the dehydrated sample with 50 mL of ethanol or methanol. The solution was stirred for 24 h in darkness. It was then centrifuged at 3,000 g for 15 min at 4 °C (Centurion® centrifuge, UK), and the supernatant was filtered through Whatman No. 1 filter paper. The extracts were used to determine polyphenols, flavonoids, flavonols, tannins, and proanthocyanidins as described below (Izuegbuna *et al*., 2019).

*Total Polyphenols*. The total polyphenols were determined in the methanolic extract using the Folin-Ciocalteu colorimetric method; 0.5 mL of extract, 2.5 mL of 10% Folin-Ciocalteu reagent was added.

The mixture was vortexed and left to stand at room temperature for 10 min. Then, 2 mL of 7.5% sodium carbonate solution was added, mixed, and incubated in a water bath at 40 °C for 30 min. After cooling, the absorbance was read at 765 nm. Results were expressed as mg of gallic acid equivalent per g of dry weight (mg GAE/g DW).

*Total Tannins*. The tannins were determined using 0.1 mL of methanolic extract, 7.5 mL of distilled water, 0.5 mL of Folin-Ciocalteu reagent, and 1 mL of 35% $Na_2CO_3$. The volume was brought to 10 mL with distilled water. The mixture was combined and left to stand at room temperature for 30 min. Absorbance was read at 725 nm. Results were reported as mg GAE/g DW.

*Total Flavonoids*. These were determined using the aluminum chloride colorimetric method. Two milliliters of distilled water, 0.5 mL of ethanolic extract, and 0.15 mL of 5% sodium nitrite solution were added. The mixture was combined and left to stand for 5 min at room temperature. Then, 0.15 mL of 10% aluminum chloride solution was added and incubated for 5 min at room temperature. After incubation, 1 mL of 4% sodium hydroxide and distilled water were added to complete a volume of 5 mL. The mixture was combined and incubated at room temperature for 15 min. The absorbance was read at 420 nm. Results were expressed as mg of quercetin per g of dry weight (mg Quercetin/g DW).

*Total Flavonols*. Two milliliters of ethanolic extract were mixed with 2 mL of 10% aluminum chloride in ethanol. Three mL of sodium acetate were added, and the mixture was incubated at 20 °C for 2.5 h. Absorbance was measured at 440 nm. The total flavonols content was expressed as mg of quercetin equivalent/g DW (mg QE/g DW).

*Total Proanthocyanidins*. Three milliliters of vanillin-methanol (4%), 0.5 mL of methanolic extract and 1.5 mL of hydrochloric acid were added. The mixture was combined and left to stand for 15 min at room temperature. The absorbance was read at 550 nm. The proanthocyanidin content was reported as mg of catechin equivalent/g DW (mg CE/g DW). The absorbance readings were taken using a spectrophotometer (Tecan®, model Infinite Pro 200, Austria).

*Total Alkaloids*. These were determined as described by Longbap *et al.* (2018) with some modifications. Briefly, 0.25 g of flour was mixed with 100 mL of 10% acetic acid in ethanol. The mixture was left to stand for 4 h at room temperature and then filtered. The recovered supernatant was reduced to a quarter of its original volume by heating at 55 °C. Concentrated ammonium hydroxide was added to complete the precipitation. The precipitate was recovered by centrifugation for 10 min at 5,000 g and washed with diluted ammonium hydroxide. The resulting residue was dried at 80 °C and weighed. The alkaloid content was calculated using the following equation [1]:

$$\text{Alkaloids (\%)} = \frac{\text{Weight of precipitate}}{\text{Weigth of sample}} * 100 \quad [1]$$

*Saponins*. Five grams of dehydrated sample were mixed with 20 mL of 20% ethanol, stirred for 30 min, and then heated at 55 °C for 4 h. The mixture was cooled and filtered. The supernatant was saved, and the residue underwent a second extraction. The two filtrates were combined, and the volume was reduced to 40 mL in a water bath at 90 °C. The concentrate was transferred to a 250 mL separating funnel, and two extractions with diethyl ether were performed. Sixty milliliters of n-butanol were added


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to the aqueous phase, and two washes with 5% sodium chloride solution were carried out. The remaining solution was heated in a water bath at 40 °C until complete evaporation and constant weight. The saponin content was calculated using the following equation [2]:

$$\text{Saponin } (\%) = \left(\frac{\text{weight of residue}}{\text{weight of sample}}\right) * 100 \quad [2]$$

*Phytates*. Two grams of flour were added to 50 mL of 2% hydrochloric acid. The mixture was stirred for 3 h and then filtered; then 25-mL aliquot of the supernatant, 50 mL of distilled water and 5 mL of 0.3% sodium thiocyanate were added. The resulting solution was titrated with an iron chloride solution containing 0.00195 g of iron $mL^{-1}$ until the development of a dark brown color that persisted for at least 5 min. The phytate content was calculated using the following equation [3]:

$$Phytate\ (\%) = Titre\ value * 0.00195 * 1.1952 * 100 \quad [3]$$

*Chlorophylls*. The concentrations of chlorophylls a and b, carotenoids, and pheophytins were determined using 1 mL of 80% acetone added to 100 mg of flour. The solution was stirred for 24 h in darkness. The solution was centrifuged for 10 min at 13,000 g, and the supernatant was filtered through a 0.45 µm filter. The absorbance of the recovered supernatants was read at wavelengths of 470, 645, 653, 654, 663, and 665 nm using a spectrophotometer (Tecan, model Infinite Pro-200, Austria). The absorbance values were used to calculate the concentrations of total chlorophyll, chlorophyll a (Cha), chlorophyll b (Chb), total carotenoids, and total pheophytins by applying the following formulas (Hynstova *et al.,* 2018) [4-8]:

$$TCh = 7.05 * A662 + 18.09 * A645 \quad [4]$$
$$Cha = 11.24 * A662 - 2.04 * A645 \quad [5]$$
$$Chb = 20.13 * A645 - 4.19 * A662 \quad [6]$$
$$Total\ carotenoids = ((1000 * A470) - (1.9 * Cha - 63.14 * Chb))/214 \quad [7]$$
$$Total\ pheophytins = (321.3 * A653) - (208.4 * A654) \quad [8]$$

***Physicochemical and functional characterization***

The conductivity and total solids (°Brix) were determined using 10% suspensions of the dehydrated sample prepared in triplicate, stirred at room temperature for 30 min, and centrifuged for 10 min at 3,000 g.

*Conductivity*. The conductivity of the recovered supernatant was determined using a conductivity meter (Milwaukee®, MW170, USA).

*Total soluble solids (°Brix).* A refractometer (Milwaukee®, MA871, USA) was used. The pH and titratable acidity were determined using 1% solution of the dehydrated sample prepared and centrifuged for 10 min at 3000 g. The supernatant was recovered, and the pH was determined using a pH meter (Milwaukee®, MW150, USA).

*Titratable acidity*. Ten milliliters of the supernatant were titrated with 0.1 N NaOH to a pH of 8.2. The acidity was reported as % citric acid, and the calculation was performed using the formula [9]:


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$$\text{Citric acid}(\%) = \frac{\text{Normality of NaOH} * \text{Titre value} * \text{mEq citric acid}}{\text{Weigth of sample}} * 100 \quad [9]$$

*Water solubility index (WSI).* This was determined using 2.5 g of flour dissolved in 30 mL of water with a glass stirrer, heated in a water bath for 15 min at 90 °C, cooled, and centrifuged for 10 min at 3,000 g. The recovered supernatant was dried at 110 °C, and the weight of the dry residue was obtained. The WSI was calculated using the following formula [10]:

$$WSI(\%) = \frac{Wdissolved\ solids\ in\ supernatant}{Wdry\ solids} * 100 \quad [10]$$

*Water holding capacity (WHC).* One gram of flour was mixed with 50 mL of distilled water, vigorously stirred for 1 min, and the suspension was centrifuged for 15 min at 10,000 g. The supernatant was discarded, and the weight of the precipitate was obtained. The WHC was expressed as g of water absorbed per g of dry weight.

*Color*. Color was determined using a colorimeter (Minolta® CR-300, Japan) based on the values for L*, a*, and b*. The L* value indicates luminosity, with 0-100 representing dark to light. The a* value indicates the degree of red-green color, with a higher positive value representing a redder color. The b* value indicates the degree of yellow-blue color, with a higher positive value representing a more yellow color.

### *Statistical analysis*

The experiments were conducted at least in triplicate. The results are expressed as mean values ± standard deviation (X±SD). The data were analyzed using SPSS V 23. Statistical software package (IBM® Corp. NY, USA).

## Results and Discussion

*Cylindropuntia imbricata* is a wild plant of little interest, which may be due to the seasonality of the plant and the difficulty in handling it. As shown in Figure 1, *C. imbricata* has a structure with protuberances, making it difficult to remove the epidermis and spines. As the plant ages, both the spines and the epidermis become thicker and harder.

**Figure 1.** Cladodes of *C. imbricata* at 3-4 months of age. The short, thick spines are visible, with some immature ones at the top. The protuberances on the cladode body are also observable.


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The plant material used for this study was 3 to 4 months old. At this stage, there is less parenchyma loss when removing spines because they are small and immature, and the epidermis is not yet very thick. The fresh cladodes of *C. imbricata* had a moisture content of 90.8 ± 1.1%, which is slightly lower than reported for some *Opuntia* species such as *O. dilenii* (92.0±1.48) (Méndez *et al*., 2015) and *O. ficus-indica*, for which different studies have reported values ranging from 92.33 ± 1.36 to 94.0 ± 0.78 (Méndez *et al*., 2015; Rocchetti *et al*., 2018).

The results of proximate analyses of the dehydrated *C. imbricata* sample obtained after milling are shown in Table 1. The moisture content was 6.6 ± 0.2%, similar to what has been reported for *O. ficus-indica* (7.73 ± 1.26) by Albergamo *et al*. (2022) and Di Bella *et al*. (2022) reported values ranging from 5.43 ± 0.073 to 7.25 ± 1.18 for different varieties of this same species, concluding that variety influences moisture content.

**Table 1**. Proximate composition of *C. imbricata* flour.

| **Variables** | **Mean values ± SD*** |
|---|---|
| Moisture of fresh cladode | 90.8 ± 1.1 |
| Moisture of dehydrated cladode | 6.6 ± 0.2 |
| ash | 20.2 ± 0.4 |
| Fat | 1.3 ± 0.1 |
| TDF | 21.3 ± 0.6 |
| Carbohydrate | 42.1 ± 3.8 |
| Protein | 5.8 ± 0.6 |

*Data are presented as mean ± standard deviation (SD), n=3. TDF, Total Dietary Fiber. Values are expressed in percentages.

The total ash content in the dehydrated *C. imbricata* sample was 20.2 ± 0.4%, higher than reported for three varieties of *O. ficus-indica*, whose values range from 12.81-15.23% (Di Bella *et al*., 2022). Albergamo *et al*. (2022) reported 18.58% for *O. ficus-indica* (L) Mill, in another study with different wild cactus species, values in a wide range from 5.2 to 19.7% were reported (Guevara-Figueroa *et al*., 2010). López-Cervantes *et al*. (2011) found values of 20.12 to 20.78% for *O. ficus-indica*, like those obtained in this study. The total ash content varies greatly among species, so differences between genera are expected. Additionally, factors such as soil composition and maturity stage influence mineral composition and concentration. However, the ash percentage of *C. imbricata* falls within the range reported for cactus pear.

*Cylindropuntia imbricata* contains 1.3 ± 0.1% total fats, within the ranges as described by Guevara-Figueroa *et al*. (2010), who reported values between 0.1 and 1.5% for different wild and commercial cactus species and varieties, and Di Bella *et al*. (2022) with values in the range of 0.76-2.46% for different cultivars. Other studies with *O. ficus-indica* report percentages of 2.30-2.38% (López-Cervantes *et al*., 2011), and 1.42-2.38% (Hernández-Urbiola *et al*., 2010), the latter conducted with cactus pear of different ages. According to all these studies, factors such as cultivar, species, and age influence fat content.

Total dietary fiber in *C. imbricata* was 21.3 ± 0.6%. Lower values have been reported for wild varieties of crystal cacti (7.1%) and peach cactus (7.7%) and similar values for the wild variety “tapon” II (20.4%) (Guevara-Figueroa *et al*., 2010), and for *O. ficus-indica* (L) Mill. (28.39%) (Albergamo *et al*., 2022).


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Another study reported values between 41.1 and 46.72% for three varieties of *O. ficus-indica* (Di Bella *et al*., 2022). The factors such as age, species, and variety, among others, influence fiber content. In this respect, *C. imbricata* is considered a potential source of fiber and can contribute to maintaining proper intestinal function.

The total carbohydrate content in *C. imbricata* was 42.1 ± 3.8%. It has been reported that there is great variation in carbohydrate content among cactus pear species and varieties. Guevara-Figueroa *et al.* (2010) reported values ranging from 42.4 to 80.9% in a study conducted with different wild cactus species. Another study with different varieties of *O. ficus-indica* reported values from 24.54 to 30.62%, also noting that season is another factor that can influence total sugar content (Di Bella *et al*., 2022). A study conducted with *O. ficus-indica* reported values of 68 g/100 g DW (López-Cervantes *et al*., 2011), so the values obtained for *C. imbricata* fall within those reported for different wild and commercial cactus pear species.

The protein content in *C. imbricata* was 5.8 ± 0.6, like some cactus pear species, for which variable values have been reported ranging from 1.48 to 11.2%, so it is not considered a protein source. In general, the proximate composition of *C. imbricata* is like that of various cactus pear species.

Regarding the physicochemical characteristics (Table 2), the pH of the *C. imbricata* solution is acidic (5.2 ± 0.2). In a study by Nabil *et al.* (2020), it was concluded that particle size influences pH. They reported a pH of 4.41 for *O. ficus-indica* in a study with dehydrated cactus pear of similar particle size to this work, suggesting that *C. imbricata* is less acidic than *O. ficus-indica*. The titratable acidity was 1.35 ± 0.21%, higher than reported for *O. ficus-indica*, whose values ranged from 0.7 to 1.09 depending on particle size (Nabil *et al*., 2020) and from 0.99 to 1.11 for domestic and export vegetable cactus (Maki-Díaz *et al*., 2015), indicating a possibly higher amount of free organic acids in *C. imbricata* than in cactus pear.

**Table 2**. Physicochemical and functional characterization of dehydrated *C. imbricata* cladodes.

| **Variables** | **Mean values ± SD*** |
|---|---|
| pH | 5.20 ± 0.20 |
| Titratable acidity (% citric acid) | 1.35 ± 0.21 |
| Soluble solids (ºBrix) | 5.50 ± 0.30 |
| Conductivity (mS) | 1.27 ± 0.36 |
| WHC (g $H_2O$/g dry weight) | 12.94 ± 0.03 |
| WSI (%) | 8.40 ± 0.20 |
| Color | L* 77.7± 1.07 |
| | a* -5.27 ± 1.11 |
| | b* 20.48 ± 0.54 |

*Data are presented as mean ± standard deviation (SD), n=3. WAC, Water absorption capacity; WSI, Water solubility index.

Cacti are generally a source of minerals. As shown in Table 3, according to EDX analysis, the composition indicates that the most abundant minerals in *C. imbricata* are calcium (25.4 ± 3.1%) and potassium (14.2 ± 3.3%), which other studies have reported as the two main minerals present in cactus pear (Albergamo *et al*., 2022; Di Bella *et al*., 2022). The presence of divalent $Ca^{2+}$ and monovalent $K^{+}$ ions play an important role in conductivity, which for this study was 1.27 ± 0.36 mS. As is known,


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conductivity depends on the quantity and type of minerals present in the sample. Other minerals present in *C. imbricata* are magnesium, aluminum, phosphorus, chlorine, and sulfur, which also influence conductivity and can perform biological functions.

**Table 3**. Mineral composition of dehydrated *C. imbricata* cladodes, determined by EDX.

| **Minerals** | **Mean values ± SD*** |
|---|---|
| C | 27.9 ± 2.7 |
| O | 32.8 ± 1.6 |
| Mg | 0.5 ± 0.2 |
| Ca | 25.4 ± 3.1 |
| Cl | 1.7 ± 0.4 |
| K | 14.2 ± 3.3 |
| Al | 0.9 ± 0.1 |
| S | 4.7 ± 0.8 |
| P | 0.16 ± 0.1 |

*Data are presented as mean ± SD, n=5. Values are expressed in percentages.

The hydration properties of *C. imbricata* were described by water holding capacity (WHC) and water solubility index (WSI). The water absorption capacity of *C. imbricata* was 12.94 ± 0.03 g of water/g of dry matter. López Cervantes *et al.* (2011) determined the effect of drying temperature on water absorption capacity, reporting values for *O. ficus-indica* from 6.44 to 14.44 g water/g dry matter, concluding that lower temperatures result in higher water retention capacity. Other factors influencing this property are particle size and cladodes age, with older cladodes having lower capacity and smaller particle size having higher water absorption capacity (Saenz, 1997). The variety has also been reported to influence this property; Ayadi *et al.* (2009) reported values of 6.85 and 3.15 $gH_2O$/g dry matter for two varieties of *O. ficus-indica*, indicating that cactus pear has a lower absorption capacity than *C. imbricata* in this case. This property is important as it shows the flour's ability to associate with water molecules, contributing to texture improvement.

The solubility index is related to the presence of water-soluble molecules, mainly sugars and polysaccharides. The WSI for *C. imbricata* was 8.40 ± 0.2%, falling between the values reported for two varieties of *O. ficus-indica* (4.78 and 25.54), whose differences depend on variety and are attributed to soluble sugar and starch content (Ayadi *et al*., 2009).

The color defined by the parameters L*, a*, and b* indicates that *C. imbricata* flour is dark green with high luminosity. It should be noted that particle size, drying technique, and plant age are factors that influence color. In a study conducted by Nabil *et al*. (2020), they found that the L* parameter positively correlates with chlorophyll and pheophytins, while the a* parameter negatively correlates with these same parameters. Additionally, b* shows a positive correlation with carotenoids. According to this report and the previously mentioned factors, the color of *C. imbricata* is influenced by pheophytins, whose presence is associated with an olive-green color.

The antioxidant properties of phytochemicals in plants are well-known. These molecules act through various mechanisms, primarily protecting against oxidative stress-induced damage. In *C. imbricata*, polyphenols, flavonoids, flavonols, proanthocyanidins, and vitamin E were found (Table 4). The


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presence of these molecules has been documented in various wild and commercial Opuntia species and varieties (Guevara-Figueroa *et al.*, 2010; Figueroa-Pérez *et al*., 2018; Kolniak-Ostek *et al*., 2020). Generally, the presence and quantity of these molecules in plants vary due to factors such as variety, maturity stage, cultivation conditions, temperature, and sun exposure, among others (Cohen & Kennedy, 2010). Overall, the quantities of these molecules found in *C. imbricata* are similar to those reported in cactus pear.

**Table 4**. Compounds in dehydrated *C. imbricata* cladodes.

| **Compounds** | **Mean values ± SD*** |
|---|---|
| Polyphenols † | 1308.70 ± 1.25 |
| Flavonoids † | 232.68 ± 2.18 |
| Flavonols † | 84.10 ± 0.15 |
| Tannins † | 133.20 ± 0.31 |
| Proanthocyanidins † | 29.52 ± 0.05 |
| Vitamin E † | 112.23 ± 0.92 |
| Saponin †† | 95.70 ± 1.10 |
| Phytate †† | 0.98 ± 0.08 |
| Alkaloid †† | 0.20 ± 0.01 |

*Data are presented as mean ± SD, n=3. The results are presented as mg of compound per 100 g of dry sample † and as percentages ††.

*Cylindropuntia imbricata* contains low amounts of tannins, which may be beneficial as tannins are considered antinutrients due to their ability to bind to dietary proteins, preventing their absorption. In adequate doses, tannins possess antioxidants, anti-inflammatory, anticancer, and antiallergic properties (Ghosh, 2015). Therefore, their presence in low quantities in *C. imbricata* may contribute to health maintenance.

The saponin content in *C. imbricata* was very high (95.7%), similar to the values reported for *O. stricta* (93.8 ± 3.43%) (Izuegbuna *et al*., 2019). However, for *O. ficus-indica* (L), a content of 0.55 g/100 g DW was reported (Al-Mushhin, 2022). Saponins are also considered antinutrients as they interfere with iron absorption. However, they also have antioxidant, anticancer, immunological, and cholesterol-lowering properties, among others.

The phytates interfere with the bioavailability of minerals such as calcium, zinc, iron, or magnesium, placing them in the antinutrient group. However, one of their major benefits is their contribution to maintaining healthy bones, in addition to being antioxidant molecules. Therefore, their presence in *C. imbricata* (0.98 ± 0.08) could be considered good from a functional perspective. Their presence has also been reported in cactus pear in quantities of 0.37% (Izuegbuna *et al*., 2019) and 0.55 g/100g DW (Al-Mushhin, 2022) slightly lower than those found in *C. imbricata*.

The alkaloids are nitrogenous compounds with pharmacological properties, making them of interest to the pharmaceutical industry. The presence of alkaloids has been reported in various cacti, including *Opuntia*, for which contents of 0.32% (Izuegbuna *et al.,* 2019) and 0.59% (Moussaoui *et al*., 2022) have been reported, higher than what was found in *C. imbricata* (0.2%). Therefore, cacti generally have low alkaloid concentrations.

As mentioned, saponins, phytates, and alkaloids are considered antinutrients. However, the quantities found in *C. imbricata* are low and thus may play a beneficial role in health.

In this study, total chlorophylls, chlorophyll a and b, as well as total pheophytins and carotenoids were determined. The obtained values are shown in Table 5. The total chlorophyll content was 21.36±0.17 mg/100 g DW, significantly lower than those reported by Ayadi *et al*. (2009) in two varieties of dehydrated cactus pear, 131.08 ± 9.36 for *O. ficus-indica f. amyclaea* and 151.78 ± 8.84 for *O. ficus-indica f. inermis*. On the other hand, values of 12.5 mg/100 g fresh weight (FW) for *Opuntia* spp. (Guevara-Figueroa *et al*., 2010) and 8.50 to 8.96 mg/100 g FW for fresh domestic and export cactus pear, respectively (Maki-Díaz *et al*., 2015), were reported.

**Table 5**. Chlorophyll, carotenoid, and pheophytin content in dehydrated cladode of *C. imbricata.*

| **Variables** | **Mean values ± SD*** |
|---|---|
| Chlorophyll a | 14.92 ± 0.18 |
| Chlorophyll b | 6.44 ± 0.02 |
| Total Chlorophyll | 21.36 ± 0.17 |
| Total Carotenoids | 4.80 ± 0.05 |
| Total Pheophytins | 89.28 ± 0.28 |
| Ratio Chla/Chlb | 2.31 |
| Ratio TC/TP | 0.24 |

*Data presented as mean ± SD, n=3. Chla: Chlorophyll a, Chlb: Chlorophyll b, TC: total chlorophyll, TP: total pheophytins. The results are expressed as mg of compound per 100 grams of dry weight.

The chlorophyll a/chlorophyll b ratio was 2.31. Although there are no optimal chlorophyll values for cactus pear, some studies have reported values for this ratio of 2.08-2.17 in fresh cactus pear (Maki-Díaz *et al*., 2015) and 2.08 for dehydrated cactus pear (Ayadi *et al*., 2009), indicating a higher content of chlorophyll a in cactus pear regardless of whether it is fresh or dehydrated. Thus, the relationship between chlorophylls a and b is maintained after the drying process.

The concentration of total pheophytins in *C. imbricata* was 89.28 ± 0.28 mg/100 g DW. Their generation may be favored by the acidic pH, as this contributes to the generation of the b fraction of pheophytin from chlorophyll b (Hsu *et al*., 2013). The chlorophylls are responsible for the green color of plants, while thermal processing or acidification causes the conversion of chlorophylls to $Mg^{2+}$-free derivatives such as pheophytins, causing a noticeable discoloration of green plant tissues to an olive-green color. The chlorophyll/pheophytin ratio was 0.24, indicating that a large part of the chlorophyll has been transformed into pheophytin, also contributing to the olive-green color of the product.

Both chlorophylls and pheophytins have been attributed anticancer, antimutagenic, anti-inflammatory, and antioxidant effects (Ferruzzi *et al*., 2002; Kang *et al.,* 2018; Pérez-Gálvez *et al*., 2020), so it is important that these molecules are not lost during the dehydration process. The carotenoids accompany chlorophyll in green tissues. The chlorophyll masks carotenoids until the tissue ages. Their importance lies in their activity as precursors of vitamin A (Meléndez-Martínez *et al*., 2004). The total carotenoid content in *C. imbricata* was 4.80±0.05 mg/100 g DW, much lower than reported for *O. ficus indica* (23.18 mg/100 g DW) (Jaramillo-Flores *et al*., 2003), and double what Nabil *et al*. (2020) reported for the same species, whose values were 1.85-2.33 mg/100 g DW. The carotenoids function

as antioxidants, capable of neutralizing free radicals that cause oxidative stress, which adds to the attributes of *C. imbricata*.

## Conclusions

The information on the physicochemical and functional characteristics of *C. imbricata* cladodes is scarce. According to the results of this study, *C. imbricata* can be a source of bioactive molecules with health applications. Furthermore, it possesses functional properties with potential for the food and pharmaceutical industries. The information generated in this work contributes to the comprehensive knowledge of *C. imbricata*, as until now, the limited available information has been mainly about its fruit and seeds. Further studies are necessary to determine the effects that may occur *in vivo*.

## ETHICS STATEMENT

Not applicable.

## CONSENT FOR PUBLICATION

Not applicable.

## AVAILABILITY OF SUPPORTING DATA

Not applicable

## COMPETING INTERESTS

The authors declare that they have no competing interests.

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## AUTHOR CONTRIBUTIONS

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