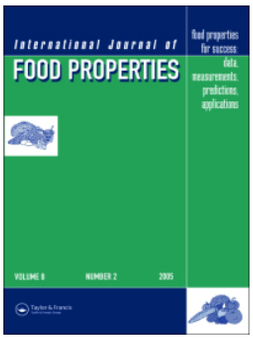

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Physicochemical and phytochemical characterization of *opuntia dillenii*: A promising source of bioactive compounds

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ABSTRACT

The objective of this ongoing research is to investigate and assess the aqueous extracts, vegetable oil, and residual plant components derived from *O. dillenii* by use of HPLC, and GC-MS. Subsequent investigations employed colorimetric techniques to identify reducing and soluble sugars. We developed and validated an energy-dispersive X-ray fluorescence (EDXRF) method for determining the concentrations of essential chemical elements (O, C, Ca, Mg, K, S, P) in seeds, juice, and peel, as well as their residues. HPLC analysis yielded results indicating that extracts from *O. dillenii* seeds, juice, and peel from distinct regions (Oujda, Nador, and Essaouira) contained elevated concentrations of metabolites. Specifically, peel and juice extracts exhibited the highest levels of organic acids and betanin compared to *O. dillenii* seeds. Conversely, seed oils displayed a noteworthy tocopherol content, predominantly δ-tocopherol. Similarly, an examination of organic acid composition in juice, peel, and seeds from three different regions revealed their elevated levels. Citric acid and oxalic acid emerged as predominant components in the juice. Overall, *Opuntia dillenii's* physicochemical and phytochemical characterization improves in understanding its potential as a bioactive chemical promoter, which could lead to the creation of new medicinal medicines or functional food ingredients.

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Introduction

Opuntia dillenii is of great economic interest because of its fruits, which are a source of valuable, biologically active, and edible vegetable oils used in cosmetology and traditional medicine.[1–3] These oils contain nutrients that are necessary for our daily diet. Plants in the genus *Opuntia* have strong environmental adaptation, growing well in high temperatures and little water availability, unfavorable circumstances for most other crops.[4] It can also be used as a meal and/or a therapeutic cure. *Cactus* pears are widely consumed as functional foods in Mediterranean countries due to their high nutritional value as well as their

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high content of several secondary metabolites (betanin, phenolic acids, flavonoids, tocopherols, sugars, and so on) with a wide range of biological activities.[5] They provide energy, are high in nutrients like essential fatty acids, phytosterols, pigments, and phenolic compounds, and can carry fat-soluble vitamins.[6,7] They are used in the food and cosmetic industries to make various products, including (margarine, chocolate, cooking oils, soaps, lotions). The fruit of *Opuntia dillenii* has been used as an unrefined antidiabetic medicine in preparing several health drinks from this plant. Scientific studies show that the fruit of *Opuntia dillenii* has antidiabetic, cholesterol-lowering, anti-inflammatory, and analgesic properties.[8] Several researchers have already been conducted to investigate the chemical composition of several cactus pear cultivars.[5] Pigment concentration, specifically betalains, determines fruit color, influencing fruit stability.[9] It is why betalains have recently gained popularity as food colorings. Several studies have evaluated the antioxidant activity of *O. dillenii* seed oil. Liu et al., demonstrated that *Opuntia dillenii* has very high antioxidant activity, and Bouhrim et al., assessed the hepatoprotective effect of *Opuntia dillenii* seed oil. The hepatotoxicity caused by tetrachloromethane (CC_{l4}) in rats was mitigated by oral administration of *O. dillenii*.[10–12]

This study endeavors to utilize aqueous extracts derived from distinct components of the *O. dillenii* plant sourced from disparate geographical locations, marking the inaugural endeavor to formulate discerning betanin extracts. Our initial inquiry is directed toward elucidating the concentration of the chromophore (betanin) within diverse *O. dillenii* fractions, acknowledging the well-established diminution of pigment concentration from peel to pulp. Within this framework, a pivotal emphasis is placed upon scrutinizing the phytochemical composition, encompassing tocopherols, organic acids, sugars, and proteins, in this cactus species – particularly notable as a byproduct of the food industry. Furthermore, the valorization of *O. dillenii* remnants subsequent to fat extraction assumes paramount significance in this investigation, serving as a pivotal determinant of their prospective utility in the realms of both the food and nutraceutical industries.

Materials and methods

Plant materials

This study used Moroccan *Opuntia* fruit collected from three regions of Morocco (Oujda (34° 41′ 12″ N, 1° 54′ 41″ W), Nador (35° 10′ 42″ N, 2° 55′ 51″ W) and Essaouira (31° 30′ 47″ N, 9° 46′ 11″ W)). The plant species was authenticated by Prof. M. Bnouham (Faculty of Sciences, University Mohammed Premier, Oujda, Morocco).[1]

Fresh species were collected in sterile plastic bags and systematically brought to the laboratory. Fresh fruit seeds were separated. The separated seeds were allowed to dry at room temperature before being cold-pressed with an oil extraction machine. Extracted seed oil and fresh pulp were packaged in a 30 mL glass bottle and stored at −4°C until use.

Opuntia dillenii, often known as the prickly pear or nopal cactus, has brilliantly colored fruits that range from deep red to purple (Figure 1). Its yellow blossoms bear large, pear-shaped purple fruits. It is quite floriferous, producing little purple figs that are lighter than the rest. The color varies widely depending on variety and growth conditions, but they are known for their striking, colorful appearance.[13]

Extraction of organics oïl from O. dillenii seeds

The oil was obtained by a mechanical extraction method (cold pressing). After cleaning the seeds, they were put under pressure to extract oil in a cold state for 45 min. The oil obtained was then mechanically filtered, stored and kept at a temperature of 4°C until use.[14]

Tocopherols profile

The determination of tocopherols of organics oïl from *O. dillenii* seeds was carried out according to the modified method of AOCS Ce 8–89 (1989), with high-performance liquid chromatography (HPLC) (Agilent technology series 1200, USA) equipped with a UV-Diode array detector. The

Figure 1. Opuntia dillenii fruit.

separation was carried out on a silica Uptisphere 120Å NH2 column with a length of 150 mm, an internal diameter of 3 mm and a porosity of 3.5 µm by a mobile phase composed of a hexane/isopropanol mixture (99/1, v/v) at a flow rate of 1 mL/min. To determine the DO, seed oil, 500 mg, was solubilized in 5 mL of hexane and 20 µL of this solution was injected into the HPLC. Identification was carried out using commercial tocopherol standards (α,β,γ-tocophérol) and Sigma-Aldrich (Saint-Louis USA). The tocopherol concentration is then calculated from the external calibration curve with different standard concentrations in ethanol (2; 4; 6; 8; 10 mg/mL) and presented in milligrams per 100 g dry matter. All calibration curves were linear (R^2 >0.99).[15,16]

Aqueous extraction process

Twenty grams of crushed seeds, juice, and peel of *O. dillenii* were macerated with 50 mL of water. The mixtures were filtered after being agitated at 20°C for 2 hours. The extracts were condensed using a rotary evaporator (Laborota 4000; Heidolph Instruments, Schwabach, Germany). Before usage, the extracts were kept in sealed, dark bottles at 4°C. For this, we envisaged evaluating the phytochemical study of the *Opuntia* fruit and using the residue for the first time to determine its nutritional value.[17]

Valorization of aqueous extract and residues of O. dillenii

To evaluate the nutritional importance of the aqueous extract and the residues obtained after the extraction process, we conducted a series of studies: an organoleptic analysis, a physicochemical study, proteins, sugars, and the mineral composition amount of *O. dillenii* samples.

Physico-chemical analysis of O. dillenii seeds and residues

The ash was determined on the analytical dry matter (seeds, juice, peel, and their residue) on 5 g of sample steamed at 105°C until the weight of the dry matter was stabilized. After obtaining the analytical dry matter, the crucibles and their contents were heated in a muffle furnace at 550°C for about 8 hours. These crucibles are then weighed after cooling in a desiccator. The calculation of the ash content is carried out as follows:

$$CT\% = \frac{P0 - P1}{P0 - P2} \tag{1}$$

where, P0-Weight of the empty crucible, P1-Weight of the crucible and oven-dried sample 105°C, P2-Weight of the crucible and calcined residue.

EDXRF analysis of samples

After calcination, the seeds, juice, and peel (before and after extraction, i.e. the residue) of *O. dillenii* samples were analyzed using a scanning Shimadzu X-ray Fluorescence Energy Dispersion Spectroscopy (EDX-7000). The X-ray source for the element Al-U was set to 50 kV 144 uA, while the X-ray source for the element NaSc was set to 15 kV 999 uA.

Reducing sugars by DNS

Reducing sugar was determined according to the method of Bernfeld (1955),[18] the principle of which is based on the reduction of DNS (3,5-dinitro-salicylic acid) (oxidant) by sugars in an alkaline medium in the presence of heating. Due to the reduced DNS, the orange-red color is measured by spectrophotometry at a wavelength of 540 nm. The reducing sugars are expressed in mg glucose equivalent using a standard range prepared from a glucose solution concentration (0.1–4 mg/mL).

Total soluble sugar content

We applied the Dubois Method to the dose of soluble sugars. 100 mg of fresh material was added to test tubes, then 2 ml of 80% ethanol. Everything was allowed to rest for 48 hours. After that, the alcohol was evaporated by immersing the tubes in a 70° water bath. After cooling, 20 ml of distilled water was poured into each test tube, 1 ml of the solution was taken, and 1 milliliter of 5% phenol was added while stirring well. Finally, 2 mL of concentrated sulfuric acid was added, and the test tubes were placed in a freezer for 25 minutes before a spectrophotometric analysis at a wavelength of 490 nm was performed.[19] The calculation is based on the equation derived from the calibration range: $Y = 76.876\ X + 0.0765$, $r2 = 0.98$.

Protein content

According to the Bradford method,[20] protein determination by spectrophotometry is based on changing the absorbance from 465 to 595 nm. 100 mg of the crushed material is dissolved with 1 mL trans-HCL buffer and centrifuged at 4000 rpm for 10 min. 100 µL of the supernatant is used with 1 mL of the working solution and after 2 minutes, read the absorbance at 595 nm. In the presence of coomassie blue, the protein extract gives a blue coloration proportional to the amount of protein. The calculation is based on the equation derived from the calibration range: $Y = 0.0086X + 0.0261$; $r2 = 0.99$.

Valorization of aqueous extract of O. dillenii using chromatographic analysis

Betanin

Betanin was separated using an Agilent 1200 column (Agilent Technologies, Palo Alto, CA, USA) linked to a diode array UV detector (Bruker, Germany). Each extract (40 mL) was injected into a Zorbax XDB-C18 column (porosity 3 µm, 100 × 3 mm; Agilent Technologies 1100 series system) with the following elution gradients: 0–15 minutes, 30% B; 15–25 minutes, 100% B; and 25–35 minutes, 30% B. A (water/0.5% acetic acid) and B (methanol) mobile phases were utilized for sample elution, with a 1 mL/min flow rate at 40°C. At 540 nm, spectrophotometric detection was carried out. Compounds were discovered by comparing the retention durations of compounds to those of legitimate standards (Betanin and Isobetanin). Calibration curves were used for quantification with different standard concentrations in ethanol (2; 4; 6; 8; 10 mg/mL), and findings were given in mg/100 g dry matter. Analyses were carried out in triplicate. All calibration curves were linear ($r2 > 0.99$).

Organic acids

The determination of the organic acid content was carried out according to Scherer et al.,[21] with some modifications. It was carried out using high-performance liquid chromatography (HPLC) 1200 series (Agilent technology, USA). Indeed, the injection volume is 20 µL. The Zorbax XDB-C18 column has a length of 150 mm, an internal diameter of 4.6 mm and a porosity of 5 µm. It is maintained at 25 C and has a diode array UV detector. The mobile phase consisted of 0.01 mol/L of the buffer solution KH_2PO_4 (pH = 2.60 adjusted with o-phosphoric acid), using an isocratic elution procedure with a flow

rate of 1 mL/min. Detection was at 210 nm for citric acid, malic acid, oxalic acid and 250 nm for ascorbic acid (250 nm due to higher absorption). Peaks were identified by their retention times by comparing the UV-visible spectra. Quantification was performed using an external standard curve with different standard concentrations in ethanol (2; 4; 6; 8; 10 mg/mL) and presented in milligrams per 100 g dry matter. All calibration curves were linear (r2 > 0.99).

Sugars profile using HPLC analysis

A quantity of 1 g of the plant was mixed, added to 4 mL of distilled water, and shaken for 30 min at room temperature. The samples were centrifuged at 4000Xg for 10 min. The supernatants were filtered through a 0.45 µm filter and placed in a vial for analysis. The contents of arabinose, glucose, fructose and sucrose were obtained using high-performance liquid chromatography (HPLC) (Agilent Technologies 1200 Series) equipped with an ELSD detector (Agilent Technologies 1200 Series ELSD), operating at a temperature of 40°C and a pressure of 3.5 bar. The separation was performed with a Spherisorb® NH2 HPLC column (5 µm, L × ID 25 cm × 3.2 mm) and a temperature of 30°C. The elution system was isocratic, consisting of Acetonitrile/Water (85/15) (v/v) with a flow rate of 1 mL/min and an analysis time of 20 min. The results were calculated using the external calibration curve with different standard concentrations in water (2; 4; 6; 8; 10 mg/mL) and presented in milligrams per 100 g dry matter. All calibration curves were linear (r2 > 0.99).

Statistical analysis

All data are presented as mean standard deviation. Multiple mean value comparisons were made using one-way parametric variance analysis (ANOVA). Using STATISTICA software (version 7.1), the degree of statistical significance of the data was determined with a probability of ($p < .05$).

Results and discussion

Tocopherols

Tocopherols, or vitamin E, are fat-soluble vitamins. The most biologically active form is tocopherol, the most abundant in the diet. Vitamin E is involved in the inactivation of reactive forms of oxygen and thus is involved in the problem of oxidative stress. Cereals and vegetable oils contain the highest levels. Tocopherols are abundant in cactus seed oils. They have properties suitable for the human body as an essential lipophilic substance. Tocopherols act as antioxidants by scavenging lipid peroxide radicals from unsaturated lipid molecules, preventing the spread of lipid peroxidation.[22,23]

Table 1 shows the results of tocopherol levels in three locations: Oujda, Nador, and Essaouira. The results are expressed in milligrams per kilogram (mg/kg) and as a relative rate percentage. In our study, we looked at the tocopherols in the seeds (oil) and determined their primary forms, specifically α-Tocopherol, γ-Tocopherol and δ-Tocopherol (Figure 2). The main component of the three tocopherols was

Table 1. Tocopherol composition of *O. dillenii* seed oils (mg/100 g).

Tocopherols	Oujda	Nador	Essaouira
α-Tocopherol	7.84 ± 0.04^{a}	4.72 ± 0.07^{b}	4.88 ± 0.23^{b}
γ-Tocopherol	22.47 ± 0.32^{a}	6.32 ± 0.25^{c}	21.59 ± 0.01^{b}
δ-Tocopherol	7.26 ± 0.47^{b}	5.22 ± 0.64^{c}	89.88 ± 0.84^{a}
Total tocopherols	37.85 ± 0.71^{b}	16.27 ± 0.58^{c}	116.31 ± 1.92^{a}
Relative rate (%)			
α-Tocopherol	20.71	29.01	4.20
γ-Tocopherol	59.37	38.84	18.56
δ-Tocopherol	19.18	32.08	77.28

Values are means ± SD (standard deviation); a,b,c indicate significant differences in each row at $p < .05$.

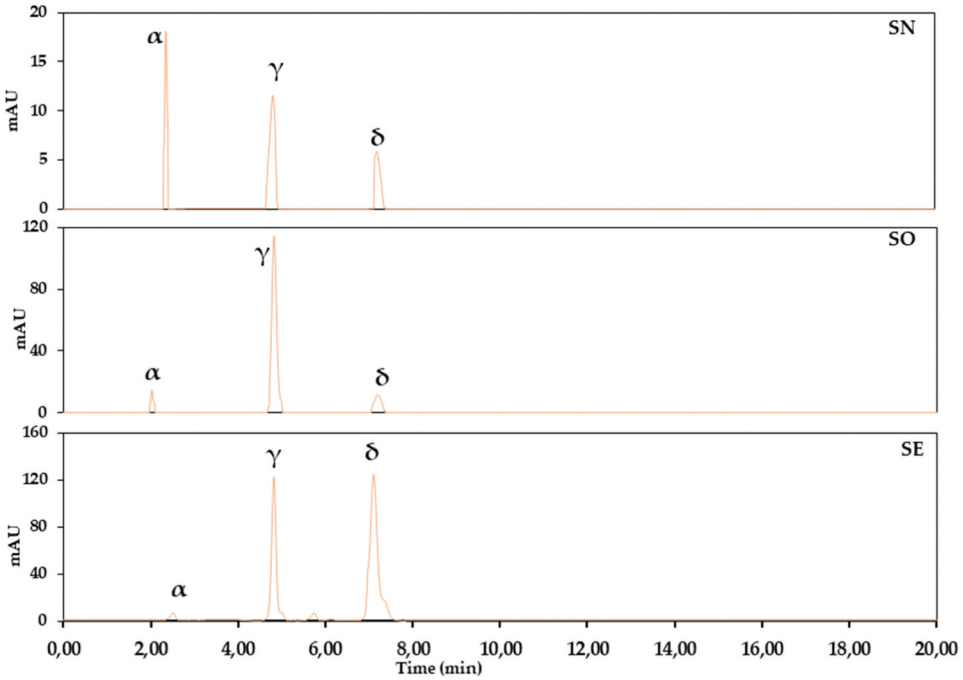

Figure 2. HPLC Chromatograms of Tocopherols (α-Tocopherol, γ-Tocopherol and δ-Tocopherol) in *O. dillenii* seeds from Nador, Oujdaet Essaouira.

δ-Tocopherol. The results show a high tocopherol level, with the oil of Essaouira seeds showing a major total level of 116.31 mg/100 g of oil. The δ-Tocopherol content was the highest at 89.88 mg/100 g, followed by Oujda and Nador seeds, which had 7.26 and 5.22 mg/100 g total tocopherol contents, respectively. α-tocopherols are found in low-value oils. According to the literature, EL Mannoubi et al., discovered that γ-Tocopherol appears to be the major component of seed oil, accounting for 94.12% of the total vitamin E content. In comparison α-Tocopherol accounted for 3.42% of the total percentage.[24] Ghazi et al., found 0.29% vitamin E in the oil *O. dillenii*.[25] Hensley et al., reported that α-Tocopherol positively impacts human nutrition and health due to its antioxidant potential.[26,27] These bioactive components are free radical scavengers and natural antioxidants.[28] A study by Nounah et al. showed that cactus seed oil was rich in tocopherols (500–680 mg/kg).[29] Chbaniet al., found that γ-tocopherol is the most significant, accounting for 90% or more of total tocopherols of Cactus (Opuntia ficus-indica) Seed Oil.[30]

Opuntia dillenii fruits contain tocopherols, which have a variety of health benefits, especially because they are antioxidant and anti-inflammatory. They perform critical functions in preventing oxidative stress, supporting cardiovascular and brain health, improving immunological function, and preserving skin and eye health. Incorporating Opuntia dillenii fruits into the diet can give these essential tocopherols, hence improving overall health and well-being.[31,32]

Physicochemical analysis of O. dillenii

Table 2 presents the chemical analysis results of *Opuntia dillenii* dry powder. It compares the percentages of various elements in different parts of the plant, including the seed, residue, juice, and peel. The table shows the percentages of elements such as oxygen (O), carbon (C), calcium (Ca), magnesium (Mg), potassium (K), sulfur (S), and phosphorus (P) in each part of the plant. It also includes the percentages of moisture, protein, soluble sugars, and reducing sugars.

These results show the composition of the seed, juice, and peel before and after extraction (R), specifically regarding various elements, moisture, protein, soluble sugars, and reducing sugars (Table 2).

Table 2. Chemical analysis of *Opuntia dillenii* dry powder.

Elements	Seed (S) (%)	Residue (S) (%)	Juice (J) (%)	Residue (J) (%)	Peel (P) (%)	Residue (P) (%)
O	–	5.90 ± 0.28	43.16± 0.33	–	42.12± 0.36	37.34± 0.53
C	–	42.12 ± 0.42	–	48.96± 0.62	5.90± 0.84	17.42± 0.92
Ca	46.83 ± 0.12^{a}	28.35 ± 0.58^{b}	20.40± 0.40	25.07± 0.83	28.35± 0.74	18.41± 0.43
Mg	30.46 ± 0.20^{a}	8.26 ± 0.30^{b}	12.75± 0.75	18.01± 0.51	8.26± 0.43	9.31± 0.35
K	7.73 ± 0.30^{a}	7.27 ± 0.38^{a}	11.33± 0.84	1.35± 0.95	7.27± 0.94	9.41± 0.43
S	–	–	–	–	–	1.16± 0.52
P	15.12 ± 0.20^{a}	8.10 ± 0.18^{b}	12.36± 0.52	6.61± 0.61	8.10± 0.93	6.95± 0.37
Cendre %	98.46 ± 0.61^{a}	98.83 ± 0.72^{a}	97.13 ± 0.07^{a}	97.09 ± 0.12^{a}	98.23 ± 0.61^{a}	98.03 ± 0.63^{a}
Moisture (%)	1.54 ± 0.93^{a}	1.17 ± 0.91^{a}	6.01± 0.87^{a}	5.50± 0.49^{a}	5.89± 0.69^{a}	5.92± 0.72^{a}
Protein (mg/100g)	257.76 ± 0.27^{a}	226.00 ± 0.62^{b}	280.54 ± 0.54^{a}	140.82 ± 0.83^{b}	225.21 ± 0.72^{a}	205.61 ± 0.69^{b}
Soluble sugars (mg/100g)	435.65± 0.92^{a}	391.45± 0.82^{b}	478.82 ± 0.23^{a}	416.471 ± 0.92^{b}	344.11 ± 0.92^{a}	308.235 ± 0.03^{b}
Reducing sugars (mg/100g)	154.10± 0.03^{a}	149.40± 0.53^{b}	267.5 ± 0.12	170.2 ± 0.61	291.4 ± 0.53^{a}	258.5 ± 0.21^{b}

S. J. P: Seeds, Juice and Peel before extraction. Values are means ± SD (standard deviation); a,b indicates significant differences in each row at $p < .05$.

Calcium (Ca) content is higher in the seeds before extraction (46.83%) than the residue after extraction (28.35%), indicating that some calcium is lost during extraction. This element is essential for the human organism and is a key element in the construction of the skeleton during growth and the maintenance of bone capital, blood coagulation, muscular and cardiac activity, and muscle contraction.

The seed residue has a high percentage of carbon (42.12%) and calcium (28.35%), while the juice residue has a high percentage of carbon (48.96%). The peel has a significant percentage of oxygen (42.12%) and carbon (5.90%). Therefore, we observe a notable potassium concentration (7.73% and 7.27% for seed and residue, respectively). Phosphorus is highest in the seed (15.12%) and residue (8.10%). These elements have various important functions in the human body. It assists in maintaining water balance, alleviates pain and muscle spasms, particularly in athletes, and can potentially lower blood pressure, subsequently decreasing the chances of developing heart disease and stroke.

Magnesium is a crucial element, accounting for around 30.46% of the body's composition, and plays a vital role. Its main attribute is its ability to provide comfort and reduce stress. Additionally, it possesses muscle-relaxing properties and boosts the immune system.

Unlike organic matter, the total ash test determines the amount of minerals in *O. dillenii* seeds because minerals do not become volatile at high temperatures. The mineral fraction of the seeds was found to be 98.83% based on the total ash content. The moisture level is estimated to be around 1.54%, which is considered low. It can be attributed to the fact that the cactus seeds, a type of drought-resistant plant, serve as storage for oil. The cactus can store a significant amount of water, making it a solution for providing water to livestock in areas where water is scarce. The high water content found in the tissues of the *Opuntia* cactus serves as a valuable liquid source for the animals.[33]

Sugars and protein content

The colorimetric method was used to quantify the bioactive compounds in these parts before and after extraction to consider the recycling and potential exploitation of the seeds, peel and juice *O. dillenii* residues. The results in Table 3 show the percentage of primary metabolite contents in different parts of the plant in two different regions., both in their natural state and as residue after extraction. The primary metabolites analyzed in this study include soluble sugar, reduced sugar and protein. In the seeds, the natural content of soluble sugar ranged from 294.71 mg/100 g in Oujda to 413.39 mg/100 g in Nador. After extraction, the residue content ranged from 228.35 mg/100 g in Oujda to 391.45 mg/100 g in Nador. Similarly, the regions' natural and residue contents of reducing sugar and protein varied.

For the juice, the natural content of soluble sugar ranged from 1427.45 mg/100 g in Oujda to 1504.50 mg/100 g in Essaouira, while the residue content ranged from 880.20 mg/100 g in Oujda to 957.26 mg/100 g in Essaouira. The same pattern was observed for reducing sugar and protein contents.

Table 3. Primary metabolite contents are expressed as a percentage of dry matter.

		Soluble sugar (mg/100g)		Reducing sugar (mg/100g)		Protein (mg/100g)	
Parties	Regions	Natural[1]	Residue[2]	Natural[1]	Residue[2]	Natural[1]	Residue[2]
Seeds	Oujda	294.71 ± 0.03^{d}	228.35 ± 0.16^{d}	187.60 ± 0.15^{b}	172.90 ± 0.33^{bc}	289 ± 0.12^{ab}	231 ± 0.36^{b}
	Nador	413.39 ± 0.19^{d}	391.45 ± 0.27^{d}	154.1 ± 0.18^{c}	149.40 ± 0.36^{c}	257 ± 0.43^{b}	226 ± 0.39^{b}
	Essaouira	347.58 ± 0.23^{d}	308.77 ± 0.38^{d}	137.6 ± 0.21^{c}	131.20 ± 0.39^{c}	323 ± 0.46^{a}	289 ± 0.42^{ab}
Juice	Oujda	1427.45 ± 0.27^{a}	880.20 ± 0.15^{c}	459.80 ± 0.24^{a}	416.50 ± 0.42^{a}	172 ± 0.49^{c}	131 ± 0.45^{d}
	Nador	1456.69 ± 0.31^{a}	907.20 ± 0.18^{c}	467.60 ± 0.27^{a}	442.90 ± 0.25^{a}	152 ± 0.52^{c}	132 ± 0.48^{d}
	Essaouira	1504.50 ± 0.35^{a}	957.26 ± 0.21^{c}	478.40 ± 0.30^{a}	437.6 ± 0.26^{a}	280 ± 0.15^{ab}	140 ± 0.51^{c}
peel	Oujda	1317.21 ± 0.39^{b}	1288.53 ± 0.24^{ab}	360.31 ± 0.33^{b}	345.90 ± 0.19^{b}	279 ± 0.18^{ab}	229 ± 0.54^{b}
	Nador	1468.50 ± 0.43^{a}	1452.19 ± 0.27^{a}	365.80 ± 0.36^{b}	371.20 ± 0.20^{b}	205 ± 0.23^{bc}	157 ± 0.57^{c}
	Essaouira	1638.92 ± 0.47^{a}	1453.88 ± 0.30^{a}	388.76 ± 0.39^{b}	344.10 ± 0.21^{b}	276 ± 0.15^{ab}	225 ± 0.60^{b}

[1]Natural: matter before extraction; [2]Residue: matter after extraction; a,b,c,d indicate a statistical difference in each major column (soluble sugar, reducing sugar, and protein) at $p<.05$

In the peel, the natural content of soluble sugar ranged from 1317.21 mg/100 g in Oujda to 1638.92 mg/100 g in Essaouira, while the residue content ranged from 1288.53 mg/100 g in Oujda to 1453.88 mg/100 g in Essaouira. The content of reducing sugar and protein followed a similar trend. From Table 3. the residues are rich in sugars and proteins. It has led us to consider using the fruit waste *O. dillenii* and food parts in different agronomic and food sectors. The variations observed in the metabolite contents between different regions and parts of the plant could be attributed to several factors, including genetic differences, environmental conditions and agricultural practices. It is important to note that these results represent a specific study and may not be generalized to other regions or plant species. Further analysis and interpretation of these results can provide insights into the nutritional and biochemical composition of the plant and its potential applications in various industries, such as food and pharmaceuticals. Several previous studies have linked changes in sugar content to lower acidity and soil.[34,35]

Organic acid content

In the case of the aqueous extracts of different parts of the fruit *O. dillenii* the examination of the results in Table 4 the analysis revealed that the fruit is particularly enriched in organic acids (malic acid, tartaric acid, oxalic acid, ascorbic acid and citric acid). The analysis revealed that the juice and peel from the three regions contained the highest organic acid levels. Oxalic acid levels in Essaouira and Oujda juices are (1675.24 and 1048.07 mg/100 g, respectively). However, only a trace amount (274.91 mg/100 g) was found in Nador juice. Oxalic acid is also abundant in peel extracts. The extract of Oujda peel has the highest percentage (937.61 mg/100 g). As a result, succinic acid is present in all aqueous extracts of *O. dillenii* with reasonable values.

Nonetheless, the juice and peel extracts are abundant, with 906.44 mg/100 g for the Oujda peel and 689.41 mg/100 g for the Essaouira juice. Only trace amounts of ascorbic acid and citric acid were found in the seeds and fruit peel of *O. dillenii*, but very high levels were found in the juice ranging from 3336.69 to 5296.95 mg/100 g. The addition of ascorbic acid as an antioxidant and citric acid as an acidifier was declared on the labels of ready-to-use beverage and juice powder products.[21] According to Brazilian legislation (ANVISA. 1998)[21] and the Food Law and Agriculture Organization (FAO/WHO. 2002).[36] The recommended daily allowance of ascorbic acid for adults is 60 and 45 mg in approximately 200 ml (one glass) of all fruit juices, respectively. Low pH values can inhibit pathogen growth and thus preserve food quality.[37] Because of its antioxidant properties. Ascorbic acid (Vitamin C) is important in food and human nutrition. According to the literature, the difference in the current results could be attributed to differences in environmental standards and genotypic conditions influencing ascorbic acid content.[28] Our findings agree with those of Gaballah et al., who discovered that Opuntia fruit contains (58.18 mg/100 g) of vitamin C.[38] Chang et al. discovered that the ascorbic acid content of the seeds is undetectable. However, the peel and pulp contain ascorbic acid (1.2 mg and 15.1 mg/100 g).[39] According to similar studies, *Opuntia* fruit contains oxalic, quinic, malic and citric acid. With citric acid being the most abundant organic acid.[40] The organic acid

Table 4. Organic acids, sugars and betanin content of aqueous extracts of *Opuntia dillenii* fruit (mg/100 g).

	Constituents (mg/100 g)	Seeds			Peel			Juice		
		Oujda	Nador	Essaouira	Oujda	Nador	Essaouira	Oujda	Nador	Essaouira
Sugars	Arabinose	18.12± 0.10^{a}	46.70 ± 0.09^{b}	23.63± 0.06^{c}	4.84 ± 0.09^{d}	nd	nd	nd	nd	nd
	Glucose	286.7 ± 0.29^{e}	324.80 ± 0.26^{d}	282.91 ± 9.25^{e}	380.73 ± 17.51^{c}	421.29± 0.08^{a}	421.44 ± 0.21^{a}	211.36 ± 0.03^{f}	200.35 ± 0.08^{f}	412.56 ± 0.04^{b}
	Fructose	313.39± 0.24^{e}	383.55 ± 0.20^{c}	288.35± 0.28^{e}	390.76 ± 0.20^{c}	480.92 ± 0.11^{a}	461.78 ± 0.32^{a}	377.05 ± 0.03^{c}	200.41 ± 0.02^{f}	430.23 ± 0.04^{b}
	Saccharose	nd	81.87 ± 0.37^{c}	53.79 ± 0.19^{c}	134.92 ± 0.62^{b}	13.60 ± 0.19^{d}	405.36 ± 0.56^{a}	1.43 ± 0.06^{d}	nd	nd
Betanin	Betanin	nd	nd	nd	nd	139.3 ± 0.10^{d}	935.1 ± 0.94^{a}	624.2 ± 0.37^{b}	286.9 ± 0.46^{c}	976.6 ± 0.31^{a}
	Isobetanin	nd	nd	nd	nd	858 ± 0.13^{a}	931.8 ± 0.10^{a}	506.0 ± 0.53^{b}	20.60 ± 0.15^{d}	105.90 ± 0.75^{c}
Organic Acids	Oxalic acid	94.82 ± 1.22^{ef}	174.44 ± 13.64^{e}	41.67 ± 1.04^{f}	937.61 ± 11.99^{b}	550.24 ± 2.67^{c}	915.21 ± 1.05^{b}	1048.07 ± 10.81^{b}	274.91 ± 0.68^{d}	1675.24 ± 7.25^{a}
	Tartaric acid	114.16 ± 1.80^{d}	311.52 ± 33.89^{c}	40.20 ± 2.43^{e}	1293.33 ± 0.81^{a}	4.28 ± 0.00^{e}	232.13 ± 17.12^{cd}	138.69 ± 3.64^{d}	279.63 ± 8.64^{c}	564.81 ± 6.87^{b}
	Malic Acid	15.42 ± 0.21^{d}	14.16 ± 1.65^{d}	18.67 ± 0.05^{d}	171.69 ± 0.82^{b}	158.43 ± 4.24^{b}	116.30 ± 0.44^{c}	154.04 ± 3.95^{b}	4.43 ± 0.00^{d}	228.06 ± 1.19^{a}
	Ascorbic acid	1.81 ± 0.03^{d}	1.39 ± 1.83^{d}	4.53 ± 94.10^{d}	19.41 ± 0.07^{c}	6.16 ± 0.11^{d}	9.80 ± 0.08^{d}	23.32 ± 0.22^{c}	112.83 ± 0.20^{a}	45.98 ± 0.74^{b}
	Citric acid	6.22 ± 0.015^{d}	22.71 ± 1.23^{d}	11.55 ± 0.28^{d}	79.46 ± 0.01^{cd}	12.38 ±0.27^{d}	129.08 ± 0.05^{c}	3336.69 ± 1.08^{b}	2374.85 ± 0.08^{b}	5296.95 ± 3.11^{a}
	Sussinic Acid	101.36 ± 1.01^{de}	353.48 ± 12.20^{c}	289.29 ± 1.44^{d}	906.44 ± 1.44^{a}	70.52 ± 0.99^{e}	179.82 ± 1.23^{d}	465.47 ± 3.07^{c}	204.04 ± 0.85^{cd}	689.41 ± 4.51^{b}

nd : not detected. Values are means ± SD (standard deviation); a, b, c, d, e, f indicate significant differences in each row at $p < .05$.

content of *Opuntia dillenii* fruits has several health benefits, including increased nutritional value and medicinal potential. These acids contain antioxidant, antibacterial, digestive, and anti-inflammatory activities, which benefit metabolic and cardiovascular health and increase mineral bioavailability. Incorporating *Opuntia dillenii* fruits into the diet might thus assist in overall well-being and the avoidance of numerous medical diseases.[41,42]

Sugar content

Sugars are important in fruit quality because they provide a pleasant taste. As a result. HPLC Table 4 and Figure 3 were used to quantify fructose, glucose, arabinose and saccharose. The predominant sugars are glucose and fructose. They are present in identical amounts in all extracts of *O. dillenii* from the three locations. Ranging from 200.35 to 480.92 mg/100 g. The peel remains dominant, containing more sugar than the other two parts. Saccharose was found in high concentrations in the fruit peel *O. dillenii* harvested in the three regions (134.92–405.36 mg/100 g) but in low concentrations in the seeds (81.87–53.79 mg/100 g). Patricia et al., reported that the profile of soluble sugars from *Opuntia* showed a slight predominance of glucose (5784 mg/100 g) with fructose and saccharose appearing as minor components.[40] They discovered that fructose is the primary sugar soluble in the pulp and grain of this fruit, with values of (138 and 71 mg/100 g). These findings are similar to those reported by Gaballah et al., who found a sugar content of 26.2% in the fruit *O. dillenii*. On the other hand, The sugar content of *Opuntia dillenii* (prickly pear cactus) fruits enhances its flavor while also providing energy. Aside from sweetness, these sugars have specific biological activity and health implications. The sugar in *Opuntia dillenii* fruits is largely used as an energy source, which adds to their flavor and nutritional value. These sugars provide a rapid energy boost, assist control of blood sugar levels, promote digestive health, and may increase the fruit's antioxidant capabilities. When ingested as part of a balanced diet, the sugars in *Opuntia dillenii* can help with general health and well-being.[43,44]

Betanin

In the context of fruit valorization *O. dillenii* it is important to note that *Opuntia* fruit juice is a rich source of dietary fiber, vitamins (B1, B2 and C), and natural coloring agents (betanin and indicaxanthin).[45] which are responsible for the fruit's intense red color.[46] Food coloring is an essential component of modern processed foods. Because of its nutritional value, betalain can be used as a natural colorant in various foods.[47] This dye is water soluble and can be used as a red-violet betacyanin or yellow-orange betaxanthin. The HPLC chromatogram of the *O. dillenii* fruit (Figure 4) revealed the presence of betanin and isobetanin with retention times of 2.99 and 3.42 min, respectively. Betanin has a shorter retention time than isobetanin (Figure 5). This difference has been explained by betanin's stronger interaction with the stationary phase.[48] The juice is high in betanin and isobetanin, with 976.6–931.8 mg/100 g. respectively (Table 4). In the case of Essaouira, the peel contained 935.1 and 931.8 mg/100 g of betanin and isobetanin, respectively. Chang et al. discovered that the bark contains the highest concentrations of betanin and isobetanin. With 15.7 and 19.2 mg/100 g fresh samples, respectively. This compound is not found in the seeds.[39] Betancourt et al., on the other hand, claim that *O. dillenii* contains a high concentration of total betacyanins (16.63 mg/100 g fresh fruit) and betaxanthins (7.55 mg/100 g fresh fruit).[49] *O. dillenii* is an essential edible source of betanin which has antiproliferative properties. Prickly pear pulp extract which contains betanin, can be used as an antibiotic in cases of digestive and urinary tract injuries, infections and inflammations. It also has a significant inhibitory effect on humanoid ovarian tumors and cervical cancer cells. The optimal pH for maximum betanin stability is between 5.5 and 5.8. Long-term exposure to oxygen, light in the presence of oxygen, high temperature and water activity affect the stability of betanin. Still, it is very stable in low humidity.[50] As a food color, betanin has many health benefits and is a valuable natural resource.

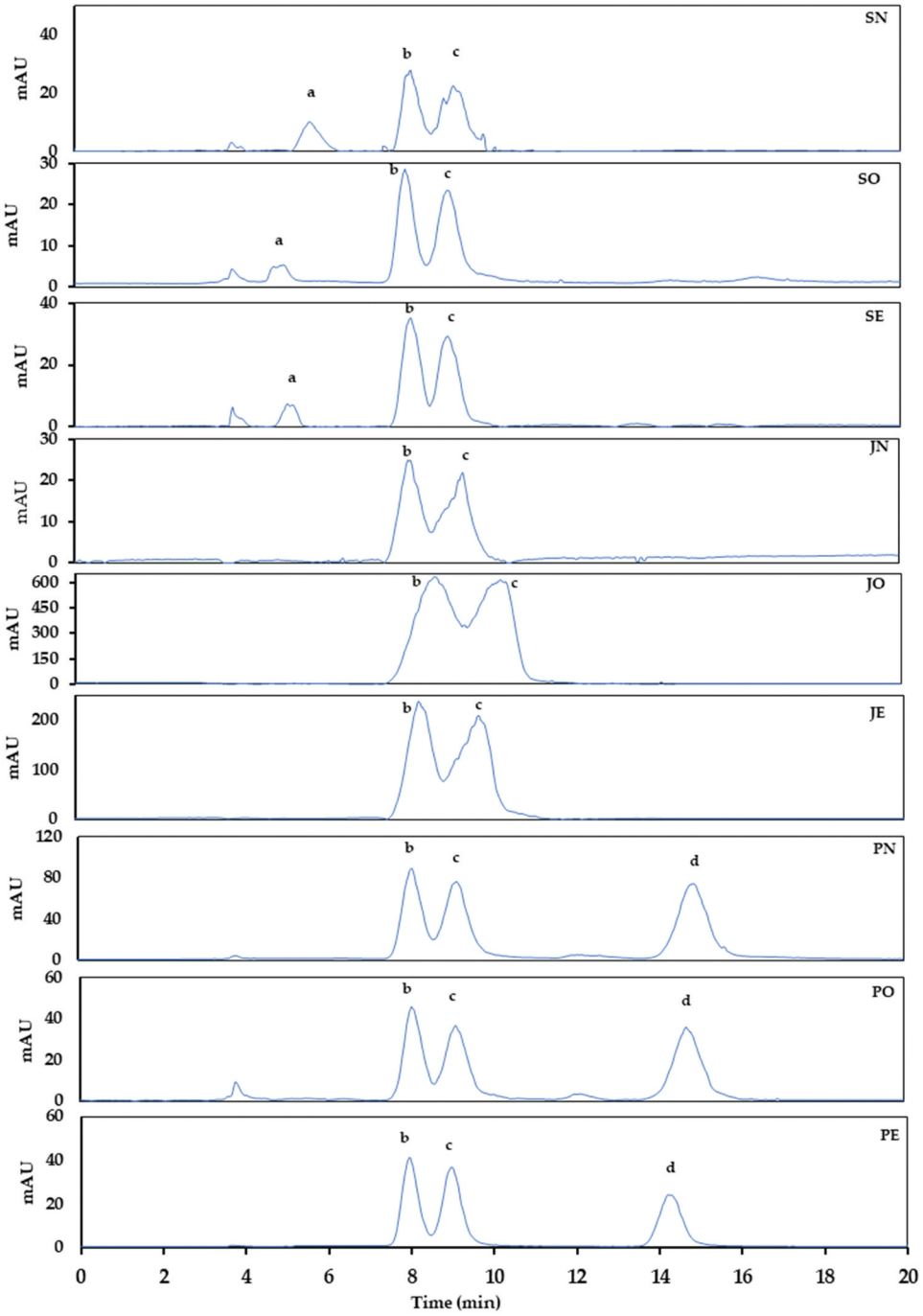

Figure 3. HPLC Chromatograms of the sugars: arabinose (a), glucose (b), fructose (c) and saccharose (d) present in aqueous extracts of *O. dillenii* seeds (S), juice (J) and peel (P) from Nador, Oujda et Essaouira.

Betanin, the primary betacyanin pigment in *Opuntia dillenii*, has several notable biological activities. It is a potent antioxidant, neutralizing free radicals and reducing oxidative stress. Betanin also demonstrates anti-inflammatory effects by inhibiting pro-inflammatory mediators and cytokines. Research shows betanin has anticancer properties, inducing apoptosis in cancer cells, inhibiting cell

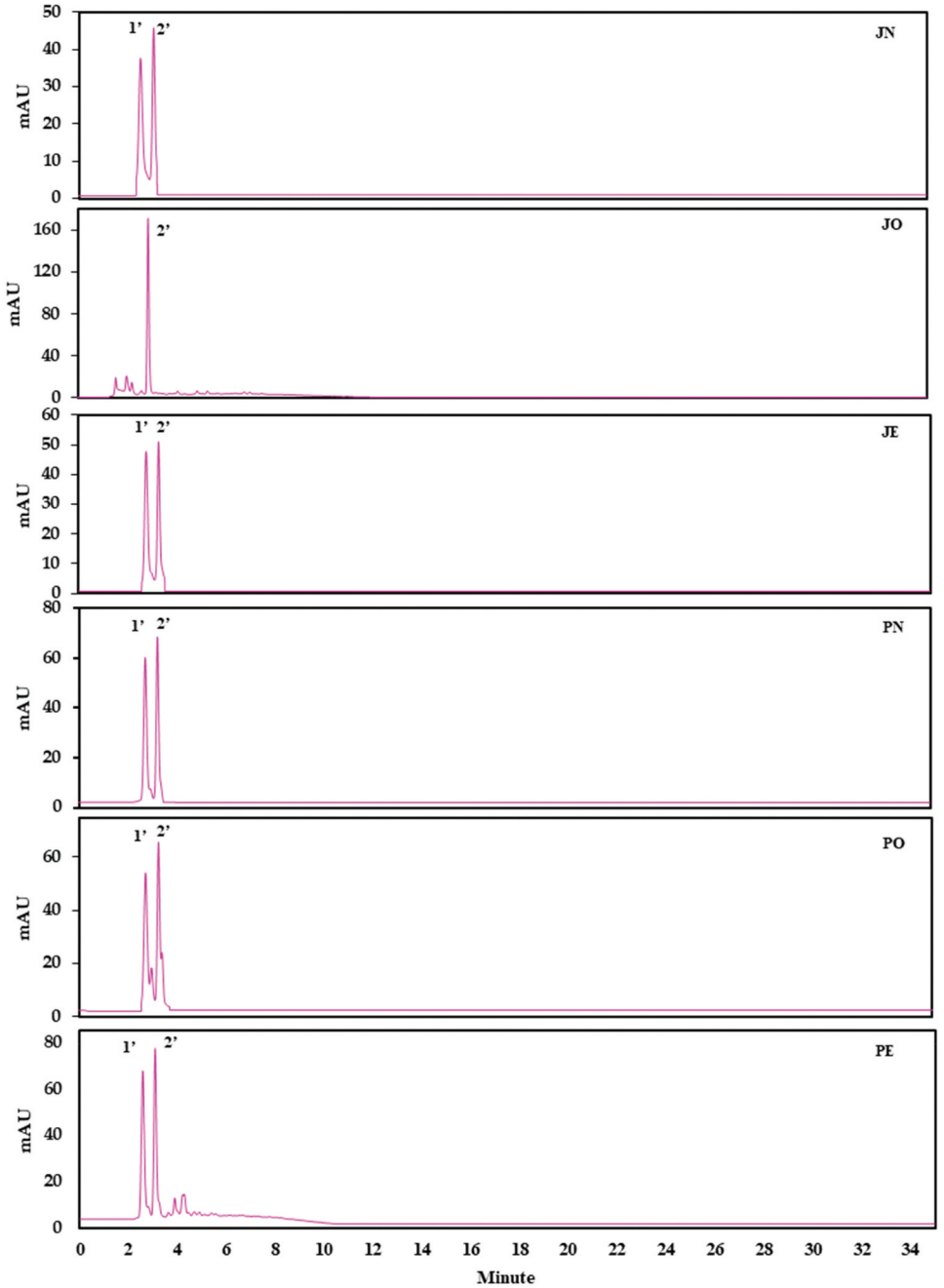

Figure 4. HPLC Chromatograms of betanin (1′) and isobetanine (2′) present in the aqueous extract of *O. dillenii* juice (J) and Peel (P).) from Nador, Oujda et Essaouira.

proliferation, and preventing metastasis. It protects the liver from toxins and oxidative stress, enhancing antioxidant enzyme activity and reducing inflammation. Betanin can improve insulin sensitivity, regulate blood sugar levels, and reduce oxidative stress and inflammation associated with diabetes. Overall, betanin has significant potential therapeutic applications due to its wide range of beneficial biological activities.[13,51]

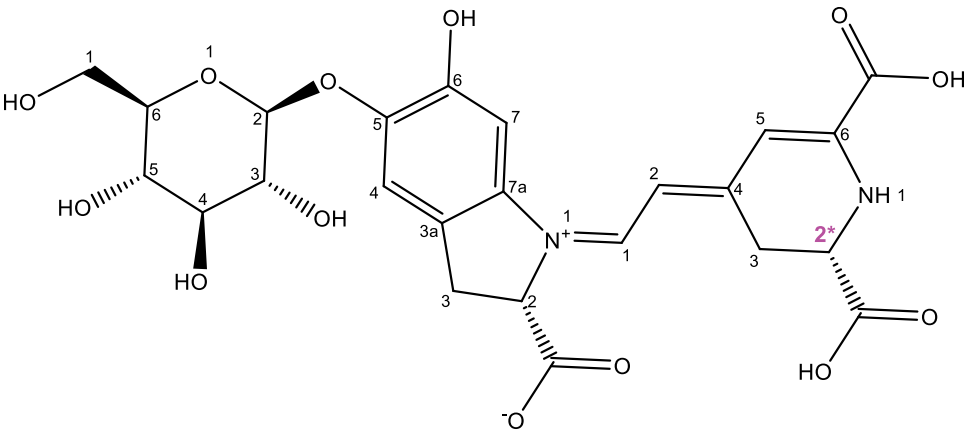

Figure 5. Betanin structure. Isobetanin: C2* epimer of betanin.

Conclusion

The examining chemical components (sugar content. organic acid betanin) of aqueous extracts of *O. dillenii* seeds, juice and peel from various regions (Oujda. Nador and Essaouira) are rich in metabolites. So, this investigation showed that the *Opuntia dillenii* seeds seed oils contain a substantial amount of tocopherol. Primarily γ-tocopherol (about 89.880.84 mg/100). Additionally, the results indicated that the peel and juice extracts were the most rich in organic acids and betanin when compared to the fruit seeds of *O. dillenii*. To further investigate the analysis of its organic acid profile, it was revealed that the juice and peel from the three regions contained the highest levels. Citric acid and oxalic acid were the primary components found in juice. The byproducts of plant processing represent an exciting and inexpensive source of polyphenols that could be used as natural, harmless and multifunctional additives with high added value.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

Author contributions

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Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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