



# Morphological and biochemical characterization of Moroccan *Opuntia dillenii* fruit: Natural source of bioactive compounds

Caracterización morfológica y bioquímica del fruto de *Opuntia dillenii* marroquí: Fuente natural de compuestos bioactivos

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## ABSTRACT

**Background:** The fruit of *Opuntia dillenii*, commonly known as prickly pear, is widely consumed for its nutritional and medicinal benefits, such as anti-inflammatory and antioxidant properties. It plays a significant role in local diets and traditional medicine, contributing to economic value. However, research on its phytochemical composition and health benefits is limited, highlighting the need for further investigation. **Objectives:** The current study assessed morphological traits, biochemical composition, and antioxidant activity using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. **Methods:** The morphological traits, biochemical parameters, and antioxidant content of the Moroccan *O. dillenii* fruit fractions, namely peel, juicy pulp, and seeds, were investigated. **Results:** The coefficient of variation of the morphological traits oscillated between 16.03 % for fruit weight and 51.83 % for seed weight, indicating a broad level of morphological variability. The total phenolic content of fruit fractions ranged from 202 to 56 g EAG/ 100 g extract, while the total flavonoids varied from 185 to 11 mg EC/100g extract. In addition, the total levels of betacyanins, betaxanthins, and condensed tannins ranged from 6.1 to 335 mg/L, 4.7 to 123 mg/L, and 12 to 8.3 mg/100g, respectively. As for ascorbic acid, it was concentrated in the juicy pulp at 580 mg/100 g, while it was absent in the seeds fraction. The phenolic compounds, flavonoids, and betalain contents were significantly correlated with antioxidant activities, whereas total ascorbic acid and condensed tannins were weakly correlated. **Conclusion:** These findings suggest that *O. dillenii* fruits may be a potential source of natural antioxidants for both food applications and medicinal functions.

**Keywords:** *O. dillenii*, morphological, biochemical, Antioxidant activity, bioactive compounds.

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## RESUMEN

**Introducción:** El fruto de *Opuntia dillenii*, comúnmente conocido como nopalera, es ampliamente consumido por sus beneficios nutricionales y medicinales, como sus propiedades antiinflamatorias y antioxidantes. Juega un papel significativo en las dietas locales y la medicina tradicional, contribuyendo a su valor económico. Sin embargo, la investigación sobre su composición fitoquímica y beneficios para la salud es limitada, lo que resalta la necesidad de una mayor investigación. **Objetivos:** En el presente estudio se evaluaron los rasgos morfológicos, la composición bioquímica, así como la actividad antioxidante mediante el ensayo DPPH. **Métodos:** Se investigaron los rasgos morfológicos, parámetros bioquímicos y el contenido de antioxidantes de las fracciones de fruto de *O. dillenii* marroquí, a saber, cáscara, la pulpa jugosa y las semillas. **Resultados:** El coeficiente de variación de los rasgos morfológicos osciló entre el 16,03 % para el peso del fruto y el 51.83 % para el peso de las semillas, lo que significa un amplio nivel de variabilidad morfológica. En cuanto al contenido fenólico total de las fracciones del fruto, osciló entre 202 y 56 g EAG/ 100 g de extracto, mientras que el total de flavonoides varió entre 185 y 11 mg EC/100 g de extracto. Además, el total de betacianinas, betaxantinas y taninos condensados osciló entre 6.1 y 335 mg/L, 4.7-123 mg/L y 12 a 8.3 mg/100g, respectivamente. En cuanto al ácido ascórbico, se concentró en la pulpa jugosa a 580 mg/100 g, mientras que estaba ausente en la fracción de semillas. Los contenidos de compuestos fenólicos, flavonoides y betalaína se correlacionaron significativamente con las actividades antioxidantes, mientras que el ácido ascórbico total y los taninos condensados se correlacionaron débilmente. **Conclusiones:** Este hallazgo sugiere que los frutos de *O. dillenii* pueden servir como fuente potencial de antioxidantes naturales tanto para aplicaciones alimentarias como para funciones medicinales.

**Palabras clave:** *O. dillenii*, morfológico, bioquímico, actividad antioxidante, compuestos bioactivos.

## INTRODUCTION

Morocco boasts a rich diversity of plant species of plant species, providing a potential source of bioactive compounds. One such species is *Opuntia stricta* var. *dillenii* (Ker. Gawler) classified within the *Cactaceae* family. This plant is native to the southeastern regions of North America, the east coast of Mexico, Bermuda, the West Indies, and northern South America (1, 2). The *Opuntia* species are cultivated in over 30 countries, covering approximately 100.000 ha. Notably, Mexico is the sole country that cultivates cladodes for commercial use on around 10.000 ha (3, 4), resulting in an impressive total production of 600.000 tons annually (5, 6).

Currently, there are more than 1500 species in the genus *Opuntia*, contributing to its wide distribution in the world, such as the Mediterranean countries, Europe, and sub-Saharan Africa (7). Most of these species are known for production of edible and aromatic fruits (8), which are notable for their acidic flavor and high seeds content (9).

Recent studies highlighted the rich chemical profile of *O. dillenii* fruits, revealing a wealth of important compounds such as 3-O-methyl quercetin, kaempferide, kaempferol, isorhamnetin, quercetin, beta-sitosterol, 4-ethoxy-6-hydroxymethyl-alpha-pyrone, opuntisterol, opuntisteroside, taraxerol, friedelin, methyl linoleate, 7-oxo sitosterol, 6- $\beta$ -hydroxystigmast-4-ene-3-one, daucosterol, eucomic acid, methyl eucomate along with polysaccharides, betalains, lipids, minerals, vitamins, and amino acids (10, 11).

This intriguing composition has secured *O. dillenii* a longstanding place in folk medicine across various cultures, evidenced by a substantial body of literature detailing its pharmacological properties (12). These properties include anti-diabetic (13), anti-gastric ulcer, anti-inflammatory (14), analgesic (15), and hypoglycemic effects (16). Additionally, the fruit juices and pulp are increasingly used as natural coloring agents in food products such as ice cream and beverages (17, 18) as well as in pharmaceuticals (17). The mucilage derived from *Opuntia* species, particularly *O. dillenii*, is also gaining recognition for its potential applications in biotechnological processes (19, 20).

The valorization and development of plant-based products have garnered considerable attention from both agri-food industry and research community. Despite this growing interest, study specifically focusing on the morphological parts of *O. dilleni* remain relatively scarce. This gap highlights the need for comprehensive research to better understand the plant's potential. To address this gap, the present work aims to: i) assess the morphological traits of the plant, ii) determine its biochemical composition, and iii) evaluate its antioxidant activity using the DPPH test. By investigating these aspects, we aim to uncover potential differences in antioxidant content and enhance our understanding of the bioactive compounds present. This knowledge could be instrumental for therapeutic and pharmaceutical applications, paving the way for the development of new plant-based products with significant health benefits.

## MATERIALS AND METHODS

### Plant material preparation of fruit fractions

In 2017, mature and healthy fruits of *O. dillenii* were carefully collected from the Aghermod region near Essaouira, the primary crop area for this plant. The juice was obtained from the pulp by filtering it through muslin cloth and then centrifuged at 5000 rpm for 10 min at 4 °C. The peels and seeds were dried and ground using an electric blender. The pulp juice, seed and, peel powder were stored at -20 °C in glass vials protected from light until used.

### Morphological characterization

In this study, 20 fruits are subjected to morphological analysis by assessing seven economically important traits, such as fruit width, length and the weight of the fruit, peel, seed, and pulp. The measurements were carried out using a digital balance and caliper. The pulp weight was calculated by the following formula: The pulp weight (g) = (Fruit weight (g) – (seed weight (g) + peel weight (g))).

### Biochemical analysis

#### Extracts preparation

The seed powder was first defatted with n-hexane for 9 hours using a Soxhlet apparatus. It was then extracted through simple maceration with 70 % ethanol for 24°C. The peel powder was subjected to extraction with 75 % ethanol with agitation for 2 hours at room temperature in the dark, followed by filtration. A second extraction was performed under the same conditions, and the two peel filtrates were combined. The seed and peel extracts were concentrated under vacuum using a rotary evaporator and then reconstituted in 10 mL of pure methanol, stored at -20°C. Additionally, 1 mL of juicy pulp was added to 10 mL of methanol, and the mixture was filtered through a 0.45 µm filter. The three extracts will be subjected to further biochemical analysis.

#### Determination of ascorbic acid content

The vitamin C was determined using the method of Benderitter *et al.* (21). A total of 500 µL of each extract was mixed with 100 µL of a solution containing 30 mg/mL of dinitrophenylhydrazine in 9 N sulfuric acid, 4 mg/mL of thiourea, and 0.5 mg/mL of copper sulfate. After 3 hours of incubation at 37 °C, 750 µL of 65 % (v/v) sulfuric acid was added.

Quantification was determined by measuring the absorbance at 520 nm and the results were expressed as mg ascorbic acid (AA) equivalents per 100 g of extract sample.

#### Determination of Betalains Content

Betalains are composed of two basic groups: red betacyanins and yellow betaxanthins. The contents of betacyanins and betaxanthins were determined according to Castellar *et al.* (22) and Stintzing *et al.* (23) with slight modifications. The ethanolic extracts of the three fractions of *O. dillenii* (juicy pulp, peel, and seeds) were diluted with McIlvaine buffer (pH 6.5, citrate-phosphate) to obtain absorbance values of  $0.9 \leq A \leq 1.0$  at their respective absorption maxima. After stirring, the samples were centrifuged with 15.000g at 10 °C for 10 min. The supernatants were then filtered through a 0.45 µm nylon Lida filter (Kenosha, WI) for spectrophotometric analysis. Betacyanins were detected at 535 nm, and betaxanthins at 484 nm, according to the following equation:

Betacyanins and betaxanthins content =  $[(A \times DF \times MW \times 1000 / \epsilon \times l)]$ .

where:

- A : Absorbance
- DF : Dilution factor
- MW: Molecular weight
- $\epsilon$  : Extinction coefficient
- l : Width of the spectrophotometer cell (1cm)

For betacyanin, the extinction coefficient is 60.000 L/ (mol cm) and the molecular weight (MW) is 550 g/mol. The extinction coefficient and MW of betaxanthin were 48.000 L/ (mol cm) and 308 g/mol respectively. The results were reported as mg/L.

#### Determination of phenolic compounds

The total phenolic compounds were determined by Folin-Ciocalteu procedure described by Singleton *et al.* (24) and cited by Wolf *et al.* (25). A total of 0.125 mL of each extract was added to 0.5 mL of deionized water and 0.125 mL of Folin-Ciocalteu reagent. After 6 min of incubation, 1.25 mL of 7% sodium carbonate solution was added, and the mixture was diluted to 3 mL with deionized water. The absorbance was measured at 765 nm, and the content of total phenolic compounds was expressed as grams of gallic acid equivalents (GAE) per gram of the extracted sample.

### Determination of total flavonoids

Total flavonoid content was measured using the aluminum chloride colorimetric method (26). Briefly, an amount of *O. dillenii* extracts were mixed with 5 mL of distilled water and 0.3 mL of NaNO<sub>2</sub> solution. Then, 3 mL AlCl<sub>3</sub> (1:10) was added after 5 minutes. Following 6 minutes of incubation, 2 mL NaOH solution was added to the mixture. The absorbance was measured against a prepared blank at 510 nm, and total flavonoids were expressed as grams of catechin equivalents (CE) per gram of the extracted sample.

### Determination of condensed tannins content

Total condensed tannins content was determined by Vermerris and Nicholson (27) and modified by Chougui and al (28). A total of 250 µL of each extract was mixed with 2.5 mL of an acidic solution of ferrous sulfate [77 mg of ferric ammonium sulfate: Fe<sub>2</sub> (SO<sub>4</sub>)<sub>3</sub> dissolved in 500 mL of (3:2 n-butanol: HCl)]. After 50 minutes of incubation at 95 °C, the absorbance was measured against a blank at 530 nm. The condensed tannins were calculated using the following formula:

$$CT = [A550 \text{ nm} \times DF \times MW / (\epsilon \times l)]$$

Where:

DF : the dilution factor

MW: molecular weight of the cyaniding (=287 g/mol)

ε : molecular extinction coefficient (=34700l/mol/cm).

The condensed tannins results were expressed as mg of Cyaniding Equivalent (CE) per 100g of extract.

### Antioxidant Activity

The antioxidant activity was tested using the DPPH (1,1-diphenyl-2-picrylhydrazyl) assay, following the method described by Campos *et al.* (29). Briefly, an appropriate amount of each extract was mixed with 2 mL of DPPH solution (6 × 10<sup>-5</sup> M in methanol). After 10 minutes, the quantification of the remaining DPPH radicals was recorded from the absorption at λ=517 nm. The inhibition of free radical DPPH (I %) was calculated as I % = [(A<sub>0</sub> - A<sub>1</sub>)/A<sub>0</sub>] × 100. Where A<sub>0</sub> is the absorbance of the blank and A<sub>1</sub> is the absorbance of the samples. The I% were plotted against the respective concentrations used. The slope of the linear portion of each graph was used to calculate IC<sub>50</sub> which is the concentration which 50% of the antioxidant activity is reduced.

### Statistical analysis

The obtained values were represented as average ± SD. Analysis of variance (ANOVA) with the LSD multiple range test at the 5% level was applied to assess the differences among means. In addition, the coefficient of variation (CV) of morphological traits was calculated using the following formula:  $CV(\%) = \frac{SD}{\bar{X}} \times 100$ . The correlation between the variables studied was analyzed using the Pearson correlation coefficient. All analysis was performed using IBM SPSS Statistics 25.0 and 26.0 with three triplicates for each parameter.

## RESULTS AND DISCUSSION

### Morphological analysis

The results of the present study revealed significant variation in the morphological characters of *O. dillenii* (Table 1). The average fruit length and width were recorded at 5±0.92 and 3±0.62 cm, respectively. These values align with the range described by Böhm (30) which reported fruit lengths of 4 to 7 cm and widths of approximately 3 cm. This consistency suggests that the morphological traits of *O. dillenii* may be influenced by both genetic factors and environmental conditions typical to its habitat.

Concerning the weight of the fruit, peel, seed, and pulp, the recorded values were 32±5.13, 10±2.11, 6±3.11 and 14±3.86 g/unit respectively. These results are lower than those reported by Diaz Medina *et al.* (31), which noted fruit weight of 40 g, 23 g for the edible portion, and 13 g for peel weight. However, they are higher than the values registered by Touil *et al.* (32), which indicated fruit weights of 25 g and 9 g for the edible portion. This variability in fruit and component weights may reflect differences in cultivation practices, soil quality, and environmental stressors across regions, emphasizing the need for context-specific studies.

The examined traits exhibited substantial coefficient of variation, indicating a high level of morphological diversity in Moroccan *O. dillenii* with a range of 16.03 % to 51.83 %. The lowest coefficient of variation was recorded for fruit weight, while the highest was observed for seed weight, which exceeded the 19.4% reported by Samah *et al.* (6) for *Opuntia* seeds. This indicates that seed weight is highly susceptible to environmental variations, which could have implications for seed development and germination rates.

In general, all characteristics exhibited coefficients of variation greater than 20%, indicating considerable variability among the parameters (33). Such significant morphological diversity suggests that there is substantial potential for selection within these traits to identify more performant genotypes. Understanding the phenotypic relationships among these traits could guide breeding programs aimed at improving specific characteristics, such as fruit size, nutritional content, or seed viability. This approach would not only enhance the economic value of *O. dillenii* but also contribute to the sustainability of its cultivation by optimizing traits that promote resilience in varying environmental conditions.

**Table 1.** Mean, standard deviation and coefficient of variation of morphological characteristics of *O. dillenii* fruits analyzed

Parameters	Means $\pm$ SD	CV (%)
Length of the fruit (cm)	5 $\pm$ 0.92 <sup>a</sup>	18.40
Width of the fruit (cm)	3 $\pm$ 0.62 <sup>a</sup>	20.67
Weight of the fruit (g)	32 $\pm$ 5.13 <sup>b</sup>	16.03
Weight of the peel (g/unit)	10 $\pm$ 2.11 <sup>a</sup>	21.10
Weight of the seeds (g/unit)	6 $\pm$ 3.11 <sup>c</sup>	51.83
Weight of pulp (g/unit)	14 $\pm$ 3.86 <sup>c</sup>	27.57

CV: coefficient of variation, SD: standard deviation

## Biochemical characterization

The results of biochemical parameters analyzed for juicy pulp, peel and seeds of *O. dillenii* fruits are depicted in the Table 2. ANOVA showed a significant difference in Betacyanin contents, with averages of 335  $\pm$  3.6 mg/L for juicy pulp, 307  $\pm$  6.1 mg/L for peel, and 6.1  $\pm$  0.6 mg/L for seeds. These values indicate the phenotypic variation within the species, which can be critical for selection in breeding programs aimed at enhancing color and nutritional quality. The Betacyanin levels found in this study are lower than the 431 mg/L reported by Stintzing *et al.* (23) for juicy purple cactus pear but are comparable to the 324 mg/L reported by Sumaya-Martínez *et al.* (34). The observed differences in Betalain content may be influenced by various factors, including environmental conditions, cultivation practices, and maturation rates at harvest (35, 36). This variability highlights the importance of phenotypic assessments for identifying superior cultivars that could thrive under specific agroindustrial conditions.

In term of vitamin C content, the highest content was observed in the juicy pulp (29.7 mg/100g), followed by the peel (4.1 mg/100g), with no vitamin C detected

in the seeds. This aligns with previous studies (20, 37) and underscores the juicy pulp's potential as a functional ingredient in the food industry. Notably, the vitamin C levels in *O. dillenii* are lower than those found in *O. stricta* (57 mg/100g) (38) and the purple variety of *O. ficus indica* (37 mg/100g) (39). This indicates a gap that could be addressed through targeted breeding programs aimed at increasing vitamin C levels, thereby enhancing the fruit's appeal in the functional food market.

The total phenolic compound content was highest in seeds (202 mg GAE/100g), followed by peel (83 mg GAE/100g) and juicy pulp (56 mg GAE/100g). This trend supports findings that phenolic content is typically higher in seeds followed by the skin and pulp components (20, 40). The levels recorded for seeds in this study exceed those reported for *Opuntia joconostle* (50 mg/100g), *Opuntia matudae* (59 mg/100g) (41) and *Opuntia ficus-indica* (48–94 mg GAE/100g) (42) for total phenolics. However, these results are lower than those reported by Tlili *et al.* (43) for *Opuntia ficus-indica* seeds, which showed a phenolic content of 268.4 mg GAE /100g.

The high phenolic content in *O. dillenii* seeds suggests considerable potential for health-related applications, including the development of nutraceuticals. This supports the hypothesis that *O. dillenii* may play a role in promoting health benefits due to its high phytochemical content, as shown in other studies focused on cactus species. In contrast, the amounts of phenolic compounds in the peel and juicy pulp are lower than those reported by Chougui *et al.* (28) for *Opuntia ficus indica* (1507 mg/100g in peel and 245 mg/100g in juicy pulp) and Moussa-Ayoub (41) for *Opuntia macrorrhiza* (760 mg/100g in peel and 310 mg/100g in juicy pulp). These discrepancies suggest that while *O. dillenii* may not currently match the phenolic profiles of some other cactus varieties, it possesses a unique phytochemical profile that could offer advantages for specific agroindustrial applications, particularly in sectors focused on natural additives.

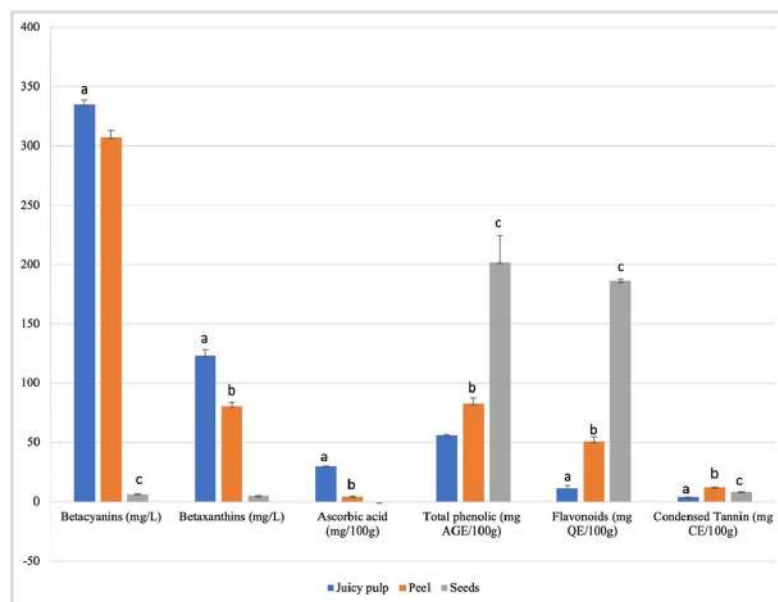
Our findings align closely with those of Wen-Chien Lu *et al.* (44), who reported a phenolic content of 117  $\pm$  10 mg/100 g in *O. dillenii* fruits. Furthermore, Gómez-López *et al.* (45) identified the predominant phenolic acids in whole *O. dillenii* fruits as protocatechuic acid derivatives (3.26  $\pm$  0.18 mg/g dry weight) and piscidic acid (0.93  $\pm$  0.00 mg/g dry weight). Protocatechuic acid is particularly notable for its extensive range of bioactive properties, which include anti-inflammatory, antioxidant,

anti-hyperglycemic, antibacterial, anti-aging, anti-angiogenic, antitumor, anti-asthma, anti-ulcer, antispasmodic, and neuroprotective effects (46).

Parallel investigations by Arba found that seeds contain elevated levels of flavonoids compared to those observed in the juice and skin of the fruit (47). This observation aligns with our findings, where the seeds extract of *O. dillenii* exhibited the highest total flavonoids content at  $186 \pm 1.453$  mg QE/100g, followed by peel at  $50.4 \pm 4.280$  mg QE/100g and the juicy pulp at  $11.2 \pm 2.599$  mg QE/100g. These results surpass those obtained by Chougui *et al.* (28) for *Opuntia ficus indica*, which reported 19 mg/100g for the peel, 50 mg/100g for the juicy pulp and 1 mg/100g for the seeds. Similarly, Ndhalala *et al.* (48) found 30 mg/100 for the peel and 25 mg/100 for the juicy pulp of *Opuntia megacantha*. The high flavonoid content in *O. dillenii* seeds suggests a potential for development in industries focused on

natural antioxidants, reinforcing the need for further exploration of the species' phenotypic traits.

Furthermore, condensed tannins were estimated in decreasing order: 12.2 mg CE/100g in peel, 8.3 mg CE/100g in seeds and 3.7 mg CE/100g in juicy pulp. These values are higher than those reported by Chougui *et al.* (28) in *Opuntia ficus indica* (1.98 mg/100g in peels, 1.16 mg/100g in juicy pulp, 0.77 mg/100g in seeds) but lower compared to those documented by Cardador-Martínez *et al.* (49) (30 to 144 mg/100g in peels and 171 to 174 mg/100g in seeds). Such variability highlights the influence of both genetic and environmental factors on phenotypic traits, particularly soil conditions and light intensity (50). Understanding these relationships can inform strategies for optimizing cultivation practices, ultimately enhancing the nutritional and commercial value of *O. dillenii* fruits in agroindustrial applications.



**Figure 1.** Biochemical content in different fractions of *O. dillenii* fruits. The letters after the mean values in each column refers to statistically different than the others ( $p < 0.05$ ).

## Antioxidant activity

The results of the antioxidant activity assessment of Moroccan *O. dillenii* using the DPPH test indicated a significant difference ( $p < 0.001$ ) among the various fruit fractions (Table 3). Specifically, the seed extract exhibited the highest DPPH inhibition, with an  $IC_{50}$  value of  $4.5 \pm 0.173$ , followed by the peel extract at  $5.8 \pm 0.190$  mg/mL and the juicy pulp at  $6.2 \pm 0.144$  mg/mL. These findings highlight the superior antioxidant capacity of the seeds compared

to the peel and pulp. Previous studies have similarly reported significant antioxidant activity in methanolic extracts of *O. dillenii*, particularly in seed extracts, which demonstrate a stronger antioxidant profile than those derived from the peel and pulp (20). In contrast, our results indicated a lower DPPH scavenging potential for the seed extract of *O. dillenii* when compared to ethanolic seed extracts ( $IC_{50} = 45\text{--}63$   $\mu\text{g/mL}$ ) (51). Additionally, another study

found that the DPPH radical scavenging activity for *O. dillenii* juice (IC<sub>50</sub> = 8.18 µL/mL) was higher than the values reported in our study (52). Despite this finding, the overall potential of *O. dillenii* remains significant, highlighting opportunities for further exploration in food preservation and functional food development. Leveraging its antioxidant properties could enhance food quality and promote health, making it a promising area for future research and industrial applications.

**Table 3.** IC<sub>50</sub> of extracts from the *O. dillenii* fruit fractions

Sample	IC <sub>50</sub> (µg/mL)
Juicy pulp	6.2 ± 0.144 <sup>a</sup>
Peel	5.8 ± 0.190 <sup>b</sup>
Seeds	4.5 ± 0.173 <sup>c</sup>

The letters after the mean values in each column are statistically different than the others ( $p < 0.05$ )

### Correlation analysis

Correlation analyses play a crucial role in breeding programs to identifying parameters that are difficult or expensive to measure (50). The Pearson correlation matrix obtained from our biochemical and phenotypic data recorded significant associations that could substantially inform breeding strategies (Table 4). Notably, the weight trait showed a significant positive correlation with antioxidant activity ( $r^2 = 0.822^{**}$ ), indicating that heavier fruits tend to exhibit enhanced antioxidant properties. This positive relationship extended to betacyanins, betaxanthins, total phenolics and flavonoids, with correlation coefficients of  $r^2 = 0.968^{**}$ ,  $r^2 = 0.975^{**}$ ,  $r^2 = 0.977^{**}$ ,  $r^2 = 0.999^{**}$  respectively. Such strong correlations highlight the interconnectedness of these compounds and their collective influence on the antioxidant potential of *O. dillenii*. Furthermore, betacyanins displayed a significant positive correlation with betaxanthins ( $r^2 = 0.958^{**}$ ), suggesting a synergistic relationship between these pigments. Conversely, both betacyanins and betaxanthins exhibited significant negative correlations with total

phenolics ( $r^2 = -0.972^{**}$ ,  $r^2 = -0.988^{**}$  respectively), and flavonoid ( $r^2 = -0.955^{**}$ ,  $r^2 = -0.983^{**}$  respectively), indicating that as these betalains increase, the levels of total phenolics and flavonoids may decrease.

In addition, ascorbic acid was significantly negatively correlated with condensed tannins ( $r^2 = -0.823^*$ ), yet it showed a positive correlation with betaxanthins ( $r^2 = 0.848^{**}$ ), emphasizing the complex interplay of these phytochemicals in influencing health benefits. Total phenolic compounds also exhibited a significant positive correlation with flavonoids ( $r^2 = 0.980^{**}$ ), further underscoring the importance of these compounds in the antioxidant capacity of *O. dillenii*. These findings align with the work of Abu Bakar *et al.* (53), which highlighted a strong relationship between DPPH free radical scavenging activity and total flavonoids content.

Notably, the predominant flavonoids in cactus fruits quercetin, kaempferol, and isorhamnetin (54) are more potent antioxidants than vitamins as phenolic compounds can inhibit prooxidant effects on proteins, DNA, and lipids by generating stable radicals (55). Our study confirms a moderate correlation between ascorbic acid content and antioxidant activity, particularly when compared to total phenolic content, suggesting that both compounds contribute to the overall antioxidant profile.

Moreover, both betacyanins, betaxanthins exhibited strong correlation with antioxidant activity, corroborating extensive research demonstrating the potent radical scavenging activity of betalains *in vitro* due to their redox potentials (56). Interestingly, the methanolic extract from betacyanin-free seeds of *O. dillenii* demonstrated a higher antioxidant capacity than extracts from peel and pulp, even when betacyanins were present in comparable concentrations (20). Finally, condensed tannin did not show a significant correlation with antioxidant activity ( $r^2 = 0.073$ ), a finding that contrasts with the results reported by Chougui *et al.* (28), suggesting that their role in antioxidant activity may be more complex or context-dependent.

**Table 4.** Correlation matrix of biochemical and phenotypic parameters analyzed

	Weight	Betacyanins	Betaxanthins	Ascorbic acid	Total phenolic	Flavonoids	Condensed Tannin
Betacyanins	0,667						
Betaxanthins	0,757	0,958**					
Ascorbic acid	0,674	0,666	0,848*				
Total phenolic	-0,570	-0,972**	-0,955**	-0,716			
Flavonoids	-0,666	-0,988**	-0,983**	-0,761	0,980**		
Condensed Tannin	-0,391	-0,124	-0,398	-0,823*	0,212	0,260	
IC <sub>50</sub>	0,822*	0,968**	0,975**	0,752	0,977*	0,999**	-0,264

\*\* . Correlation is significant at the 0.01 level, \* . Correlation is significant at the 0.05 level.

## CONCLUSION

The data indicate that *O. dillenii* exhibits considerable morphological variability, especially in fruit weight, alongside a noteworthy concentration of antioxidant compounds. Specifically, phenolic compounds were predominantly found in the seeds, while betalains comprising betacyanins and betaxanthins, along with ascorbic acid, were primarily concentrated in the juicy pulp. In contrast, condensed tannins were most abundant in the peel fraction. These findings suggest that the entire fruit possesses significant potential as a natural antioxidant and could serve as a valuable additive in functional foods. Furthermore, this information provides a solid foundation for breeding programs aimed at enhancing crop yield and quality for this species, potentially leading to the development of improved cultivars with higher antioxidant properties.

## CONFLICT OF INTEREST

The author reports no conflict of interest.

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## AUTHOR'S CONTRIBUTION

**Sofia ZAZOULI:** Conceptualization of research work and design of experiments; Execution of sampling and laboratory experiments, Interpretation of data, Writing and preparation of the manuscript.

**Ghizlane KABIRI:** Statistical analysis and interpretation, Preparation of the manuscript.

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