

Research Article

Comparative Analysis of Antioxidant Potency and Phenolic Compounds in Fruit Peel of *Opuntia robusta*, *Opuntia dillenii*, and *Opuntia ficus-indica* Using HPLC-DAD Profiling

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The edible part of the prickly pear fruit is most commonly consumed, while the peel is often discarded, leading to the loss of valuable bioactive components, such as phenolic compounds. This research aims to assess the antioxidant activity, total phenolic content, and quantities of phenolic and flavonoid contents of fruit peel of *Opuntia ficus-indica*, *Opuntia robusta*, and *Opuntia dillenii*. Ten phenolic compounds were identified, with isorhamnetin being the most abundant flavonoid measuring 7184.09 $\mu\text{g/g}$, while sinapic acid was identified as the major phenolic acid measuring 1806.36 $\mu\text{g/g}$. *Opuntia robusta* exhibited the highest total phenolic content, total flavonoids, and total phenolic acids measuring 5583.19 mg GAE 100 g⁻¹, 21041.03 $\mu\text{g/g}$, and 3265.17 $\mu\text{g/g}$, respectively. *Opuntia robusta* and *Opuntia dillenii* demonstrated the higher antioxidant capacity in scavenging 2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) compared to *Opuntia ficus-indica*, whereas *Opuntia dillenii* and *Opuntia ficus-indica* exhibited the highest concentration of antioxidants capable of reducing the Fe³⁺ complex of ferric ions (TPTZ)³⁺ to the ferrous complex Fe (TPTZ)²⁺. In contrast, the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and total antioxidant capacity (TAC) assays showed no significant difference between the examined species. Compared to the local species *Opuntia ficus-indica*, the two newly introduced *Dactylopius opuntiae*-resistant species are richer in phenolic compounds and exhibit greater antioxidant activity.

1. Introduction

Nopal cactus, commonly referred to as prickly pear, belongs to the cactaceae family, comprising around 2000 distinct species [1]. According to [2], Mexico, Italy, and South Africa are the largest producers and consumers of prickly pear, accounting for 45%, 12.2%, and 3.7% of the world's production, respectively. This versatile plant serves various purposes, such as fodder for livestock, pharmaceutical, and also, its fruits and young cladodes are highly suitable for human consumption [3]. Furthermore, the cactus has also applications in the industry and medicine. This drought-resistant perennial plant provides economic benefits especially to rural communities. The fruits and young cladodes

can be sold in local markets, providing an additional source of income for farmers. Consequently, prickly pear emerges as an excellent choice for preserving livelihoods, alleviating poverty, and generating employment opportunities due to its properties [4]. According to a report by the Food and Agriculture Organization (FAO) and the International Center for Agricultural Research in Arid Zones (ICARDA), cultivating prickly pear cactus can enhance resilience to drought and soil impoverishment, addressing challenges faced by rural communities [5]. In Mexico, the pear sector presents significant employment potential, particularly in arid regions, with approximately 20000 families relying on this agriculture [2]. Cultivating cactus pears proves to be an effective strategy for combating climate change and

promoting rural development, especially in arid and semi-arid areas [2].

The fruit is the most consumed and appreciated component in the prickly pear plant, earning widespread recognition across the globe. In some regions, particularly in North America, young cladodes claim place in consumption, in which the young cladodes are frequently prepared by grilling or broiling [6]. The low fiber content in prickly pear cladodes makes them easily decomposable, contributing to their reputation as substantial methane producers [7]. The edible portion of the fruit is the most used part of the plant, whereas the fruit peel is rarely used except as livestock feed (Potgieter and Mulaudzi). Either the consumption or industrial processing of the fruit generated a large amount of solid waste, which is mainly fruit peel. Currently, the issue of food waste has escalated to a significant global concern, emerging as a recent focal point within academic discussions [8]. The predominant disposal method involves burying food waste in landfills, resulting in the loss of its energy content. In fact, waste landfilling continues to be viewed as a viable waste management solution in numerous countries worldwide. Unfortunately, this inadequate management of food waste has profound and detrimental effects on the environment, particularly contributing to climate change [9]. Apart from environmental pollution, the disposal of waste is progressively becoming more costly.

Many studies primarily focus on the characteristics of the fruit's edible portion, often overlooking by-products, such as peels, which constitute 44.51% of the fruit [10]. However, there should be a growing interest in harnessing by-products generated from industrial processing or human consumption [11]. In effect, the disposal of fruit peel could lead to loss of valuable components like phenolic compounds, which possess a wide range of biological activities, such as anticancer, cardioprotective, anti-inflammatory, antimicrobial, and antioxidative [12]. In the last three decades, the determination of biological activity, especially antioxidant activity, has gained attention of researches [13]. Additionally, an oxidative process can generate free radicals, which has a harmful effect on health, such as hypertension, cancer, atherosclerosis, cardiovascular diseases, nitrosative stress, diabetes mellitus, and neurological disorders [14]. Consequently, antioxidants and especially natural substances exhibiting antioxidant properties have attracted the attention of scientific community [15].

Polyphenols, categorized as secondary metabolites predominantly present in plant tissues, are synthesized through shikimic acid and phenylpropanoid pathways [16]. These polyhydroxy phytochemicals, characterized by aromatic compounds containing at least one phenol group in their structure, represent the most prevalent secondary metabolites in plants, boasting over 8000 identified structures [17]. Based on their chemical structures, phenolic compounds are divided into various subclasses like flavonoids, phenolic acids, coumarins, curcuminoids, quinones, tannins, lignans, and stilbenes [16]. For the past few years, polyphenolics become a subject of interest in a scientific fraternity and many investigations were elaborated on these compounds. Epidemiological studies have demonstrated that the

consumption of beverages and foods rich in phenolic content can reduce the risk of heart disease by acting as antioxidants against low-density lipoprotein [18]. Numerous investigations within the scientific community have focused on the bioactivities of polyphenolics, revealing their diverse properties, including anti-inflammatory, antimicrobial, antioxidant, and antiproliferative activities [19]. Presently, there is heightened interest among researchers and food manufacturers in the antioxidant properties of phenolics and their potential preventive role in various oxidative stress-related conditions [20]. The shift toward exploring natural sources of antioxidants has gained momentum, particularly with the elimination of synthetic antioxidants in food applications. Nutritionists have recently directed their attention to the nutritional and health benefits of polyphenols, recognizing their capacity to influence oxidative stability, color, bitterness, odor, astringency, and flavor [16, 21]. Various conventional and nonconventional methods are employed to extract phenolic compounds. The most commonly used traditional extraction techniques include liquid-liquid extraction, solid-liquid extraction (Soxhlet extraction), and maceration, whereas the alternative techniques encompass multiple methods, such as supercritical fluid extraction, ultrasound-assisted extraction, microwave-assisted extraction, pressurized liquid extraction, and countercurrent chromatography.

The spectrophotometric assessment of antioxidant efficacy is carried out by measuring the ability of biological sample to scavenge the synthetic-colored radical. Two kinds of assays can be performed, the first based on hydrogen atom transfer, while the second, of particular interest to us, encompasses electron transfer, including DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2'-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid), and FRAP (ferric reducing antioxidant power) assay. These chemical assays are commonly used due to their simplicity, cost-effectiveness, easy accessibility, and independence from advanced laboratory equipment [22].

To the best of our knowledge, rare studies have investigated the phenolic compounds and antioxidant activity of fruit peel, particularly for the species *O. dillenii* and *O. robusta*. Most research on fruit peel has focused only on *O. ficus-indica*, which creates a research gap. The objective of the present contribution is firstly to highlight the importance of valorization peel fruit by studying its components, and this involves examining total phenolic content, phenolic compounds, and conducting antioxidant tests (DPPH, FRAP, TAC, and ABTS) as potential reservoirs of natural antioxidants. The statistical exploration of the correlation between phenolic content and antioxidant activity was also undertaken. Secondly, it is to compare the local species (*O. ficus-indica*) with the two recently introduced resistant species to the *D. opuntiae* (*O. robusta* and *O. dillenii*).

2. Materiel and Methods

2.1. Plant Material. The research study concentrated on three distinct groups of populations collected from the Oujda region in the northeastern part of Morocco (Spring

2023), located at coordinates 34°41' 12.001" N and 1° 54' 41" W. This geographic area is known for its Mediterranean climate, characterized by cool winters, hot summers, and irregularly distributed precipitation [23]. Healthy fruits used in the current study (intact of infection and infestation) were selected and carefully cut at their bases from the chosen plant.

2.2. Sample Preparation. The fruits were subjected to a completely cleansing with tap water, and the peels were separated manually from the rest of the fruit. Subsequently, the peels were cleaned and shade-dried at room temperature and finely ground to powder suitable for extraction. Then, oven drying occurred in an oven drier maintained at a consistent temperature of 30°C for a duration of 48 hours. The resulting dried samples were then processed in an electric grinder (Type ME 2 B, 800 W; INTERMITTENT 15 s/1 min; manufactured in France). Finally, the resulting powder was preserved in an air-tight bottle.

2.3. Extraction and Determination of Total Phenolic Content. As per reference [24], phenolic compounds were extracted by combining 0.1 g of powder with 5 mL of 50% acetone (v/v). Subsequently, the acquired mixture underwent vortexing for a period of 10 minutes and was then subjected to centrifugation at 5000 rpm for 15 minutes. This extraction procedure was iterated three times to guarantee the complete extraction of phenolic compounds. Then, a filtration using a 45 µm microfilter was carried out. For the assessment of total polyphenol content, 50 µL of the obtained supernatant was used. Absorbance measurements were conducted with a RAYLEIGH UV1800 Ultraviolet-Visible spectrophotometer (Beijing Rayleigh Analytical Instrument Corporation, China) at a wavelength of 760 nm. Utilizing gallic acid as a reference standard, a calibration curve was established, and the outcomes were presented as milligrams of gallic acid equivalent per 100 grams of dry matter (mg GAE100 g⁻¹·DM).

2.4. Antioxidant Activity Determination. The evaluation of antioxidant capacity across diverse species under study involved employing various methodologies focused on neutralizing free radicals. These methods included determining total antioxidant capacity (TAC), the [2,2-di(4-tert-octylphenyl)-1-picrylhydrazyl] (DPPH) assay, an evaluating ferric reducing antioxidant power (FRAP) and the 2,20-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) test. The outcomes were measured in milligrams of Trolox equivalent per Gram of dry matter (mg TE g⁻¹ DM). Optical density measurements were conducted using a UV-Vis spectrophotometer, specifically the RAYLEIGH UV1800 model.

2.4.1. Total Antioxidant Capacity Assay. The determination of total antioxidant capacity (TAC) followed the procedure outlined in [25]. The phosphomolybdenum reagent was created by mixing equal amounts of 4 mM ammonium molybdate, 28 mM sodium phosphate, and 0.6 M sulfuric

acid. The experimental process involved mixing 1.5 mL of this reagent with 50 µL of the extract, followed by thorough vortexing and an incubation period in a water bath at 95°C for 90 minutes. Subsequently, absorbance was promptly recorded at 695 nm, with a blank used as a reference for baseline correction. The results were expressed in milligrams of Trolox equivalent per Gram of dry matter (mg TE g⁻¹ DM).

2.4.2. Radical Scavenging Activity of 1,1-Diphenyl Picrylhydrazyl—DPPH Assay. The assessment of DPPH free radical scavenging activity in the samples was conducted using spectrophotometry, following the outlined procedure in reference [26]. This assay relies on the antioxidant's electron transfer ability to neutralize the DPPH radical, causing a measurable color change, which is quantified at 517 nm. There is a reciprocal relationship between the absorbance of the reaction mixture and the extent of DPPH free radical scavenging activity. In this study, 50 µL of each sample was introduced into 2 mL of a 0.13 mM methanolic solution of DPPH. The resulting mixture was vigorously vortexed and then incubated in a dark environment at room temperature for 30 minutes. The outcomes were presented in milligrams of Trolox equivalent per Gram of dry matter.

2.4.3. Ferric Reducing Antioxidant Power (FRAP) Assay. The ferric-reducing antioxidant power (FRAP) assay is based on the measurement of an antioxidant's ability to reduce ferric ions (Fe³⁺) and form an intensely blue ferrous complex (Fe²⁺), as described in reference [25]. In this procedure, each sample, comprising 50 µL, was mixed with 0.65 mL of 0.2 M phosphate buffer (pH 6.6) and 0.65 mL of a 1% ferricyanide solution. After 20 minutes of incubation at 50°C in a water bath, the reaction was stopped by adding 0.65 mL of 10% trichloroacetic acid. Following this, 0.65 mL of the extract was combined with 0.65 mL of water and 0.25 mL of a 0.1% solution of FeCl³⁺. The reducing power of the tested antioxidants can be assessed spectrophotometrically, where increased absorption at 700 nm indicates strong antioxidant activity. The results are expressed in milligrams of Trolox equivalent per Gram of dry matter (mg TE g⁻¹ DM).

2.4.4. ABTS [2,2'-Azinobis-(3-Ethylbenzothiazoline-6-Sulfonic Acid)] Free Radical Scavenging Activity Assay. The evaluation aimed to assess the ability of antioxidants to counteract the stable radical cation, specifically 2,2-azinobis-(3-ethylbenzthiazolin-6-sulfonic acid) (ABTS•+). This radical cation presents as a blue-green chromophore with its highest absorption peak at 734 nm, and the presence of antioxidants leads to a reduction in the intensity of this chromophore.

To generate the ABTS•+ radical, 2.45 mM potassium persulfate was mixed with 7 mM of ABTS [2,2'-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid)], and the mixture was allowed to incubate at room temperature in the absence of light for 16 hours. Following this, the working solution, composed of 50 µL of the extract mixed with 2 mL of the

ABTS•+ solution, underwent a 30-minute incubation at room temperature in a light-shielded environment. Finally, absorbance was measured at 734 nm using a spectrophotometer, with a blank used as a reference [25]. The results are stated in milligrams of Trolox equivalent per Gram of dry matter (mg TE g⁻¹ DM).

2.5. HPLC-DAD of Phenolic Compounds. Chromatographic analyses were performed using an Agilent 1260 Infinity II high-performance liquid chromatography system equipped with a diode array detector. The chromatographic separation utilized an Eclipse XDB-C18 column (3.5 µm particle size, 150 × 4.6 mm internal diameter, USA). The elution solvent consisted of a mixture of water (A) and acetonitrile (B), with the addition of formic acid (1%, v/v) in water. The chromatographic separation employed a gradient elution process, starting at 2% B and held constant from 0 to 6 minutes. Subsequently, it increased to 12.5% B between 23 and 33 minutes, further rising to 30% B from 33 to 38 minutes, then to 45% B from 38 to 42 minutes. This was followed by a stepwise increase to 75% B from 42 to 47 minutes and maintaining 100% B from 47 to 49 minutes. The solvent composition reverted to 2% B from 49 to 50 minutes and remained constant at 2% B from 50 to 51 minutes. The flow rate was set at 0.6 mL/min, with a 10 µL injection volume. Extract separation was monitored at multiple wavelengths (254, 280, 300, and 340 nm), and UV/visible spectra for each compound were recorded over a range from 190 to 800 nm. To identify the compound within the sample, we examined the retention time of the detected peaks and the UV spectra, comparing them with the standards from Sigma-Aldrich in Saint-Louis, United States. Quantification was conducted using an external standard curve of isorhamnetin (20–200 µg/mL), chlorogenic acid (20–200 µg/mL), sinapic acid (20–200 µg/mL), gallic acid (6.25–100 µg/mL), kaempferol (6.25–100 µg/mL), ferulic acid (8–60 µg/mL), and hydroxycinnamic acid (8–60 µg/mL) based on their respective commercial standards. The HPLC profiles were visualized and analyzed using the Agilent OpenLab CDS software. Results are expressed as milligrams of standards per 100 grams of dry matter [27].

2.6. Statistical Analysis. In this study, all analyses of the prickly pear cladode were conducted in triplicate to ensure the robustness and reliability of the findings. A one-way analysis of variance (ANOVA) was performed to identify variations among the means of different samples. Statistical significance was considered established when the *p* value was below 0.05, indicating a statistically significant difference. Following this, the Tukey post hoc test was employed to conduct multiple comparisons of means, allowing for a more detailed assessment of differences among groups. The results are presented as the mean ± standard deviation, and the statistical analysis was carried out using IBM SPSS Statistics 25 software. Additionally, correlations among the obtained data were calculated using Pearson's correlation coefficient (*r*), providing insights into the relationships between the variables under consideration.

3. Results

3.1. Total Phenolic Content of Fruit Peel. The assessment of total phenolic content (TPC) was conducted for each of *O. robusta*, *O. dillenii*, and *Opuntia ficus-indica* (Figure 1). The results revealed significant variations (*p* < 0.02) among the species. *Opuntia robusta* exhibited the highest TPC, measuring 5583.19 milligrams of gallic acid equivalent per 100 grams of dry matter (mg GAE100 g⁻¹ DM). While *O. dillenii* displayed moderate TPC results at 5513.55 mg GAE100 g⁻¹ DM, *O. ficus-indica* showed the lower results, measuring 5464.97 mg GAE 100 g⁻¹ DM (Supplement Table 1). This indicates noteworthy differences in the phenolic content among the three *Opuntia* species.

3.2. Assessment of Antioxidant Activity of *Opuntia robusta*, *Opuntia dillenii*, and *Opuntia ficus-indica* Fruit Peel. The use of four distinct techniques aims to comprehensively evaluate the antioxidant activity and measure the antioxidant capacity of fruit peels of the various species studied. This multifaceted approach allows for a comprehensive understanding of how antioxidant properties vary among different plant species.

3.2.1. DPPH Radical Scavenging Activity. The DPPH radical scavenging activities of the species under investigation ranged from 1.19 to 1.2 milligrams of Trolox equivalent per Gram of dry matter (mg TE g⁻¹ DM) (Supplemental Table 2). Notably, no significant difference was observed between the studied species (Figure 2). In this specific antioxidant test, *O. robusta* and *Opuntia dillenii* exhibited a superior antioxidant capacity but without any significant difference with *Opuntia ficus-indica* with exhibited 1.19 mg TE g⁻¹ DM.

3.2.2. ABTS Free Radical Scavenging Activity Assay. The ABTS values varied from 0.3 to 0.38 mg Trolox equivalent per Gram of the sample, as detailed in Supplemental Table 2. Importantly, significant variations in antioxidant activity were observed among the examined species (Figure 2). *O. robusta* and *O. dillenii* demonstrated the greatest antioxidant activity, with 0.37 and 0.38 milligrams of Trolox equivalent per Gram of dry matter (mg TE g⁻¹ DM), respectively. Notably, there was no significant difference between the antioxidant activities of *O. robusta* and *O. dillenii*. Conversely, *O. ficus-indica* displayed the lowest antioxidant activity, registering 0.3 mg TE g⁻¹ DM.

3.2.3. Ferric-Reducing Antioxidant Power (FRAP) Assay. The ferric-reducing capacities of the species under investigation ranged from 0.49 to 0.83 milligrams of Trolox equivalent per Gram of dry matter (mg TE g⁻¹ DM) (Supplemental Table 2). Notably, significant differences were observed between the studied species (*p* < 0.05). Without any significant difference, *O. dillenii* and *O. ficus-indica* exhibited the highest antioxidant capacity registering 0.83 and 0.76 (mg TE g⁻¹ DM), respectively, whereas *O. robusta*

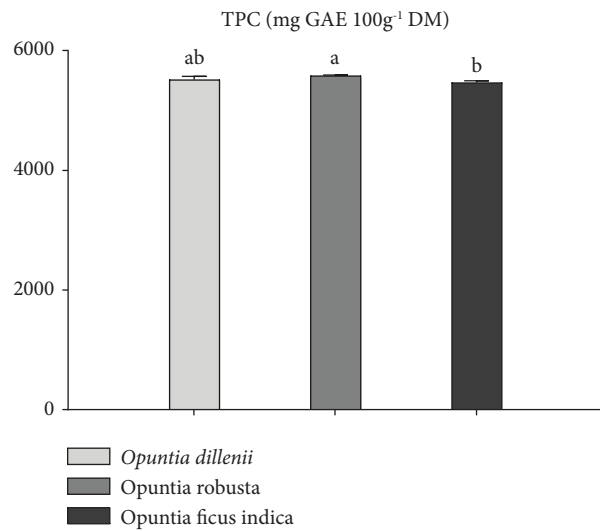


FIGURE 1: Total phenolic content of *O. ficus-indica*, *O. robusta*, and *O. dillenii* fruit peels. According to the analysis of variance, means followed by the same letters are not significantly different ($p \leq 0.05$). Error bar: standard error.

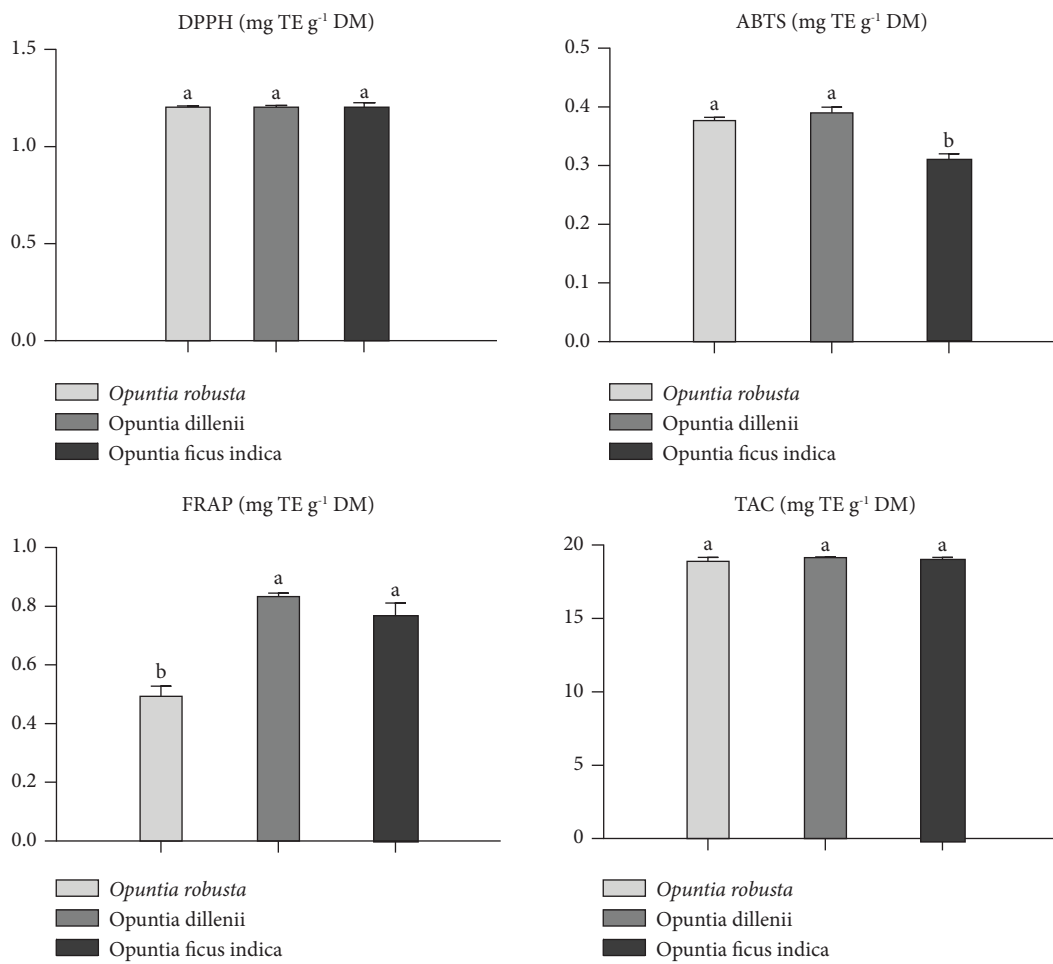


FIGURE 2: Antioxidant assays (DPPH, ABTS, FRAP, and TAC) of *O. robusta*, *O. dillenii*, and *O. ficus-indica* fruit peels. According to the analysis of variance, means followed by the same letters are not significantly different ($p \leq 0.05$). Error bar: standard error.

exhibited the lowest ferric reducing capacities, registering 0.49 (mg TEg⁻¹ DM) (Figure 2).

3.2.4. Total Antioxidant Capacity (TAC) Assay. The obtained results of TAC assay varied between 18.9 and 19.16 milligrams of Trolox equivalent per Gram of dry matter (mg TEg⁻¹ DM) (Supplemental Table 2). *O. dillenii* demonstrated the highest value at 19.16 mg TEg⁻¹ DM, followed by *O. ficus-indica* with a recorded value of 18.98 TEg⁻¹ DM. Conversely, *O. robusta* showed the lowest value with 18.9 mg TEg⁻¹ DM. However, no significant differences were observed between the species under examination (Figure 2).

3.3. Quantification of Phenolic Compounds by HPLC-DAD. In the examination of phenolic compounds, ten distinct compounds were identified, encompassing seven flavonoids and three phenolic acids (Table 1). HPLC analysis allows the identification of isorhamnetin as the most abundant flavonoid, with levels ranging from 2429.737 to 7184.09 µg/g. Following closely were quercetin and rutin. Naringenin represented the least abundant flavonoid, measuring from 95.40 to 242.83 µg/g. *O. robusta* displayed the highest concentration of four flavonoids, including isorhamnetin, epicatechin, quercetin, and rutin, while *O. dillenii* showed the highest concentration of two flavonoids: kaempferol and naringenin. On the other hand, *O. ficus-indica* demonstrated the highest concentration of only gallicocatechin.

In contrast to flavonoids, only three phenolic acids were identified, namely, sinapic acid, ferulic acid, and hydroxybenzoic acid. Sinapic acid represents the most abundant phenolic acid, with levels ranging from 592.17 to 1806.36 µg/g, followed by hydroxybenzoic acid, which ranged from 761.65 to 1563.49 µg/g. Ferulic acid, in contrast, constituted the lowest concentration ranging from 80.14 to 222.86 µg/g. Regarding the total phenolic acids, *O. robusta* displayed the highest results measuring 3265.17 µg/g, followed by *O. dillenii* with 3054.56 µg/g, while *O. ficus-indica* exhibited the lowest concentration of total phenolic acids with 1451.89 µg/g.

Concerning the total phenolic compounds, *O. robusta* demonstrated the highest concentration measuring 24306.2 µg/g, followed by *O. dillenii*, which exhibited moderate results with 16738.33 µg/g, while *O. ficus-indica* showcased the lowest content of total phenolic compounds measuring 11336.96 µg/g.

4. Discussion

Due to the limited research conducted on fruit peel of *Opuntia* species, we have focused primarily on comparing the results among the different species studied. The acquired results reveal significant differences among the studied species ($p < 0.024$). *O. robusta* exhibited the highest total phenolic content, measuring 5583.19 mg GAE 100 g⁻¹ DM. The substantial increase in polyphenolic components in *O. robusta* emphasizes the nutritional importance linked to this particular species. Considering that the examined

species were collected from the same location, indicating identical environmental conditions, it confirms that the noted distinctions among these species mainly arise from their inherent biochemical characteristics.

Our results show that the total phenolic content (TPC) of the studied species is notably higher than the values reported in previous studies [28] which documented levels ranging between 785 and 1759 mg GAE 100 g⁻¹ DM in *O. ficus-indica*. Also, our findings are so higher than those reported by [29, 30] where the total phenolic content of *O. ficus-indica* peel was reported as 1078 and 1439 mg GAE 100 g⁻¹ DM, respectively. Compared to [31] who used cyclohexanone for the extraction, declared a TPC of 5111 mg GAE 100 g⁻¹ DM for the fruit peel of *O. ficus-indica*, our results are slightly higher. This slight difference could be attributed to the environmental conditions or the method of extraction, in which they used cyclohexanone while we used acetone for the extraction. According to [29], the extraction efficiency of polyphenols from plant tissue depends on the solubility of the phenolic compounds in the chosen solvent, and higher amounts of phenol compounds are extracted with more polar solvents. It is worth noting that the TPC content of fresh peel of *Opuntia megacantha* was reported to be much lower, with 243.79 and 226.2 mg GAE 100 g⁻¹ DM, respectively [32, 33]. The observed lower TPC content could be related to the studied species, the extraction method, and also the nature of the tissue. We assume that more polyphenols could be extracted from dried tissue compared to fresh tissue.

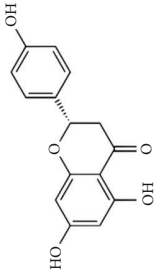
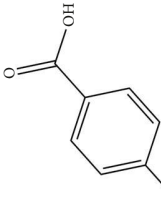
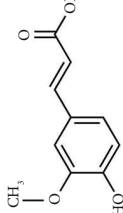
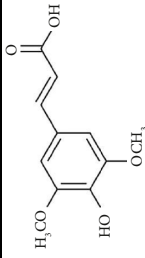
The oxidation process can occur through various pathways, such as thermal oxidation, autooxidation, enzymatic oxidation, and photooxidation, with many of these involving free radicals. Among the numerous approaches to control oxidation, the use of antioxidants proves to be the most effective, convenient, and economical method [34]. Antioxidants are substances that, when present in very low concentrations, can delay, control, or completely prevent oxidative processes. They encompass free radical scavengers, metal ion chelators, peroxide inactivators, inhibitors of pro-oxidative enzymes, and singlet oxygen quenchers [34]. Consequently, the inhibitory effect against oxidation processes manifests through various mechanisms. For that, to assess antioxidant activity accurately, a range of assays employing different mechanisms should be employed, including single electron transfer (ET), hydrogen atom transfer (HAT), metal chelation, and reducing power. As emphasized by [35], evaluating antioxidant ability necessitates the use of multiple in vitro antioxidant methods, relying solely on one method does not provide an accurate reflection of antioxidant activity. At least three different methods should be conducted simultaneously to determine antioxidant activity comprehensively.

To assess the antioxidant activity of the fruit peel of the studied species, four bioanalytical assays were employed, namely, DPPH, TAC, ABTS, and FRAP. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) removal assay, which assesses antioxidant capacity, stands out as one of the most commonly employed techniques. It is characterized by its speed, simplicity, and cost-effectiveness when compared to alternative

TABLE 1: Identification and quantification of phenolic compounds of *O. robusta*, *O. dillenii*, and *O. ficus-indica*.

N	RT (min)	Phenolic compounds	Structural formula	Flavonoids		
				<i>O. ficus-indica</i> (µg/g)	Fruit peel (µg/g) <i>O. robusta</i> (µg/g)	<i>O. dillenii</i> (µg/g)
Flavonoids						
1	11.032	Isorhamnetin		2429.73	7184.09	6571.72
2	13.60	Kaempferol		181.43	236.72	303.84
4	18.87	Gallocatechin		3194.26	1420.25	2404.88
5	18.97	Epicatechin		583.37	669.81	448.38
6	20.21	Rutin		1350.78	5440.53	2131.25
9	24.06	Quercetin		1915.04	5994.19	1580.83

TABLE 1: Continued.

N	RT (min)	Phenolic compounds	Structural formula	<i>O. ficus-indica</i> (µg/g)	Fruit peel (µg/g) <i>O. robusta</i>	<i>O. dillenii</i> (µg/g)
10	25.20	Naringenin		230.43	95.40	242.83
Total flavonoids				9885.06	21041.03	13683.77
Phenolic acids						
3	16.55	Hydroxybenzoic acid		761.65	1235.94	1563.49
7	22.21	Ferulic acid		98.07	222.86	80.14
8	22.49	Sinapic acid		592.17	1806.36	1410.92
Total phenolic acids				1451.89	3265.17	3054.56
Total phenolic compounds				11336.96	24306.2	16738.33

Values in bold represent the total flavonoids, total phenolic acids, and total phenolic compounds.

testing approaches. The DPPH test measures the transfer of hydrogen atoms from antioxidants to the free radical DPPH, resulting in the radical reduction and the formation of hydrazine (DPPH-H). This reaction is accompanied by a color change from violet to yellow that consequently can be monitored by spectrophotometry, which correlates with the antioxidant capacity. Conversely to the reported results [36], which indicated that the highest antioxidant activity was measured by the ABTS assay, followed by DPPH and FRAP test, the current study demonstrates that the DPPH assay exhibits the highest antioxidant capacity, followed by FRAP and ABTS assay.

Regarding the antioxidants capable of proficient in quenching the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, no significant differences were recorded between the studied species. All the examined species measured almost $1.2 \text{ mg TE g}^{-1} \text{ DM}$ of DPPH. The DPPH assay necessitates the dissolution of the radical in an organic solvent. Consequently, this method finds its primary applicability in the evaluation of hydrophilic antioxidants [37]. This method is not restricted to a specific antioxidant; rather, it extends to assessing the overall antioxidant capacity of the sample. As indicated by the obtained results, no significant variations were noted. Therefore, within the group of investigated species, the studied species demonstrates a consistent concentration of hydrophilic antioxidants.

Polyphenols are recognized as important natural reducing agents due to their redox properties (donation of a hydrogen atom and/or an electron to free radicals, singlet oxygen quenchers, and metal-chelating potential), leading to break the chain reaction of oxidation. Subsequently, a correlation analysis was conducted between the DPPH test and the total phenolic content (Supplemental Table 3). In the case of *O. dillenii* and *O. robusta*, moderate positive correlations were recorded, with values of 0.56 and 0.40, respectively, while, for *O. ficus-indica*, fort correlation was registered between polyphenols and antioxidant capacities measured by DPPH measuring 0.99. This strong correlation underscores a direct and pronounced connection between phenols and their capacity to scavenge the DPPH free radical. This finding aligns with the conclusions drawn by a prior study conducted by [38], which also highlighted the substantial scavenging effects of phenolic compounds against the DPPH free radical.

The FRAP assay, rapidly performed, inexpensive, and robust, does not require specialized equipment. In addition, it demonstrates high reproducibility and highest correlations with total phenolics, which make it an appropriate assay for determining antioxidant activity [39]. The FRAP assay distinguishes itself from other methods as it does not involve free radicals; instead, it focuses on monitoring the reduction of ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}) [32]. Moreover, to ensure iron solubility and promote electron transfer, the FRAP assay is conducted under acidic pH conditions (pH 3.6). This adjustment enhances the redox potential, leading to a shift in the dominant reaction mechanism [40].

In contrast to the DPPH test, the FRAP assay enables differentiation among the studied species. *O. dillenii* exhibited the highest concentration of antioxidants capable

of effectively reducing the Fe^{3+} complex of ferric ions (TPTZ^{3+}) to the ferrous complex $\text{Fe}(\text{TPTZ})^{2+}$. Following closely, *O. ficus-indica* also demonstrated substantial reducing power. Conversely, *O. robusta* displayed the lowest antioxidant activity among the studied species. The antioxidant properties are mainly attributed to the presence of phenolic compounds, indicating that the effectiveness of a fraction is proportional to its phenolic concentrations [41, 42]. A strong correlation of 0.89 was registered between polyphenols and antioxidant capacities measured by FRAP assay in *O. robusta*. Meanwhile, moderate and lower positive correlation was recorded for *O. dillenii* and *O. ficus-indica*, respectively (Supplemental Table 3). The FRAP assay showed a lower correlation between phenolic content and the antioxidant capacity compared to DPPH and ABTS. Our results contrast with those reported by [39], who asserted that the correlation between total phenolic contents and antioxidant capacity is stronger when employing the FRAP assay compared to the DPPH or ABTS assay. Although the FRAP assay is effective and reproducible, the results can vary based on the analysis time for the reaction between antioxidants and Fe^{3+} , which can range from a few minutes to several hours. Consequently, a single-point absorption endpoint may not completely capture the reaction, as different antioxidants may necessitate different reaction times for detection [43, 44]. Therefore, it is recommended to complement the FRAP assay with other methods to identify dominant mechanisms for different antioxidants [45].

The ABTS test stands out as a crucial method for assessing antioxidants in samples. In contrast to the other assays, the ABTS test is characterized by its applicability, which differs from the two previously discussed tests: DPPH and FRAP. In effect, the ABTS assay enables the assessment of both the hydrophilic and hydrophobic antioxidant capacity [46, 47]. In addition, the ABTS antioxidant assay is applicable across a broad pH range. The obtained finding from the ABTS test differed from the second assays previously discussed assays (DPPH and FRAP). *O. robusta* and *O. dillenii* demonstrated a higher antioxidant capacity in scavenging 2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) than *O. ficus-indica*, which displayed the lowest antioxidant capacity. This observed difference could be attributed to the characteristic of ABTS assay, which allows to gauge both hydrophilic and hydrophobic antioxidants. Our results are lower than those documented by [48], who stated that the ABTS values range from 1.6 to $9.26 \text{ mg TE g}^{-1} \text{ DM}$ of Spanish *O. ficus-indica* cultivars. As our study comports *O. ficus-indica*, the observed difference may be attributed to the variations in the solvents employed and also to the edaphoclimatic conditions. Notably, a strong positive correlation was observed between the total phenolic content and the ABTS assay of *O. dillenii*. Additionally, a moderate to strong positive correlation was found between the total phenolic content and the ABTS test of *O. robusta*. In contrast, low correlation was observed between the total phenolic content and the ABTS of *O. ficus-indica*.

Concerning the phosphomolybdenum assay (TAC), no significant difference was observed between the examined species. The TAC assay assesses the antioxidant capacity in

both fat-soluble and water-soluble antioxidants, highlighting the distinctions between TAC and other examined antioxidant assays. Regarding the correlation between the studied species and total phenolic content, a strong positive correlation was evident for *O. ficus-indica* (Supplemental Table 3), while *O. robusta* and *O. dillenii* displayed a moderate positive correlation.

According to [49, 50], the antioxidant activity is influenced by the solvent utilized, agricultural practices, and geographic location. However, in our investigation, the samples were collected from identical locations, and the same assays and a uniform experimental protocol were applied to all the species under scrutiny. Therefore, the observed variations among the examined species can be predominantly attributed to genetic factors.

Phenolic compounds, the most extensively distributed secondary metabolites, originate biogenetically from either the shikimate/phenylpropanoid or the “polyketide” acetate/malonate pathway [51]. Phenolic compounds, boasting more than 8000 identified structures, stand out as the most prevalent secondary plant metabolites. Recently, researchers have shown heightened interest in these compounds due to their intriguing bioactivities, including anti-inflammatory, antioxidant, antiproliferative, and antimicrobial properties [14, 17].

According to the findings, *O. robusta* stands out as the species with the higher concentration of phenolic compounds, closely followed by *O. dillenii*. In contrast, *O. ficus-indica* exhibits the lowest level of these compounds. It is crucial to emphasize that all the examined species were collected from the same geographic region and underwent a consistent extract preparation procedure, underscoring consistent edaphoclimatic conditions. Therefore, the observed variations in secondary metabolites cannot be attributed to external factors like abiotic or biotic influences but are mainly linked to the inherent biochemical characteristics of the species under investigation.

In investigating phenolic compounds, a direct comparison with other species has been conducted, mainly due to the limited number of studies conducted on the peel of prickly pear species, particularly *O. dillenii* and *O. robusta*. Concerning the phenolic acids, the HPLC analyses allow the identification of ferulic acid, sinapic acid, and hydroxybenzoic acid. Our finding differs from those obtained by [31], who conducted a study on *O. ficus-indica* and reported that the main constituent is catechin followed by gallic acid, caffeic acid, chlorogenic acid, and ellagic acid. Regarding flavonoids, seven compounds were identified, including isorhamnetin, kaempferol, epicatechin, gallocatechin, naringenin, quercetin, and rutin. According to the same author previously mentioned, only three flavonoids were identified, including quercetin, kaempferol, and rutin. The observed differences could be attributed to the method of extraction and also to the edaphoclimatic conditions.

Flavonoids are an important class of natural products that are broadly distributed throughout the plant kingdom, with approximately 4000 flavonoids identified in the plants [52], and are classified as low-molecular-weight secondary metabolites with a polyphenolic structure. Now, flavonoids are recognized as an essential component in various

applications, including pharmaceutical, cosmetic, nutraceutical, and medicinal [53, 54].

Isorhamnetin, having a polyphenolic structure and specifically classified as a flavonol, is a direct metabolite of quercetin and exhibits a broad range of pharmacological activities [55]. Isorhamnetin is an immediate metabolite of quercetin, with a polyphenolic structure and is specifically categorized as a flavonol, possesses extensive pharmacological activities. Isorhamnetin possesses a wide range of pharmacological effects on neurodegenerative, tumors, cardiovascular diseases, and pharmacodynamics against pulmonary fibrosis and hyperuricemia [56–58]. In the present study, isorhamnetin emerges as the predominant flavonoid, ranging from 2429.73 to 7184.09 $\mu\text{g/g}$. The substantial quantity of isorhamnetin identified in the fruit peel adds significant value to the utilization of this by-product.

Quercetin is one of the abundant flavonoids found with levels ranging from 1580.83 to 5994.19 $\mu\text{g/g}$. Quercetin is a common flavonol found mostly as glycosides, with a sugar group linked at the 3-position by a glycosidic bond [59]. Quercetin is recognized for its diverse biological effects, which encompass antitumor and anti-inflammatory properties in malignant cancer cells. Recent research indicates that quercetin has the potential to modify the morphology and trigger apoptosis in gastric cancer cells [60].

Rutin was also found in higher amount ranging from 1350.78 to 5440.53 $\mu\text{g/g}$. Rutin (3,3',4',5,7-pentahydroxyflavone-3-rhamnoglucoside, depicted in Figure 1) is a crucial nutritional component, also referred to as rutoside, quercetin-3-rutinoside, and sophorin. The “rutin” name is derived from the plant *Ruta graveolens*. Rutin has exhibited various pharmacological activities, including vasoprotective, antioxidant, neuroprotective, cytoprotective, cardioprotective, and anticarcinogenic effects [61].

The term “phenolic acids” typically refers to phenolic compounds with a single carboxylic acid group [62]. They are categorized into two subgroups: hydroxybenzoic and hydroxycinnamic acid [63]. Hydroxycinnamic acid compounds are frequently formed as simple esters with glucose or hydroxycarboxylic acids [64]. Phenolic acids are synthesized through the phenylpropanoid pathway from shikimic acid, as by-products during the monolignol pathway, and some are produced by microbes and through the breakdown of cell wall polymers like lignin [65]. Dietary phenolic acids, present widely in plants, play a crucial protective role under oxidative stress conditions [66]. The content of phenolic acids is influenced by the extraction method, which, in turn, is regulated by factors, such as the sample-solvent volume ratio, pH, temperature, and the number and duration of each extraction step [67, 68]. Conversely, the observed variations among the studied species are primarily attributed to genetic factors.

5. Conclusion

The current study provided information about antioxidant properties and bioactive compounds of *O. robusta*, *O. dillenii*, and *O. ficus-indica* fruits peel. *O. robusta* registered the highest total phenolic compounds, which plays an

important role against oxidation, as well as the highest antioxidant activity by ABTS method. Among the four tests of antioxidant activity, ABTS and FRAP assays demonstrate that they are better estimates of the antioxidant capacity. In contrast, no significant difference was recorded between the studied species using DPPH assay and TAC assay. Consequently, the applicability of the antioxidant test to both hydrophilic and lipophilic antioxidants is found an important factor. Consequently, various assays should be applied for testing the antioxidant properties. The noted distinctions among these species mainly arise from their inherent biochemical characteristics. Concerning the newly introduced species, *O. robusta* and *O. dillenii* were demonstrated that they have the more richness of phenolic compounds compared to the local *O. ficus-indica*. In addition, it showed important antioxidant capacity in all performed assays. The peel of the studied species shows that it contains important amounts of phenolic compounds, which can be used in various fields, such as pharmaceutical and cosmetic and food industries. However, additional research is necessary to investigate other vital components within these species, such as fatty acids and minerals. This exploration would contribute to a more comprehensive understanding of their nutritional and medicinal capabilities [44, 45, 69].

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Supplementary Materials

Supplementary Material, Table 1: Total phenolic content of *Opuntia ficus-indica*, *Opuntia robusta*, and *Opuntia dillenii* fruit peel. Supplementary Material, Table 2: Antioxidant tests (DPPH, ABTS, FRAP, TAC) of *Opuntia robusta*, *Opuntia dillenii*, and *Opuntia ficus-indica* fruit peel. Supplementary Material, Table 3: Correlation between total phenolic content and the antioxidant assay of *Opuntia robusta*, *Opuntia dillenii*, and *Opuntia ficus-indica* fruit peel. Supplementary Material, Figure 1: HPLC-DAD chromatogram of phenolic compounds of *Opuntia dillenii* fruit peel. Phenolic compounds: (1) isorhamnetin, (2) kaempferol, (3) hydroxybenzoic acid, (4) gallo catechin, (5) epicatechin, (6) rutin, (7) ferulic acid, (8) sinapic acid, (9) quercetin, and (10) naringenin. (Supplementary Materials)

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