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Total Polyphenol Content, Antioxidant Activity and Phytochemical Constituents of Hydroethanolic Extract of Fruits and Seeds of *Opuntia dilleniid (Ker Gawl)* Haw. Cultivated in Tunisia

Farah Zidi[1], Amel Azaza[1], Monia Bendhifi Zarroug[2], Abdallah Fraj[1], Mouna Ben Farhat[3] and Kheiria Hcini[1,2*]

[1]University of Gafsa, Faculty of Sciences of Gafsa, Department of Life Sciences, University Campus, Sidi Ahmed Zarroug, 2112 Gafsa, Tunisia

[2]Biodiversity, Biotechnology and Climate Change Laboratory (LR11ES09), Department of Life Sciences, Faculty of Science of Tunis, Tunis El Manar University, 2092, Tunisia

[3]Laboratory of Aromatic and Medicinal Plants, Borj Cédria Biotechnology Center, BP 901, Hammam-Lif 2050, Tunisia

ABSTRACT

Opuntia dillenii (Ker Gawl) Haw. have been widely used due to their nutritional composition and beneficial effects on health and are now used as medicinal plants in various countries. In this study, we investigated the phytochemical constituents and the antioxidant potential of fruits and seeds hydroethanolic extracts of *Opuntia dillenii* L. cultivated in Tunisia. The highest values of total phenolic and flavonoid contents (TPC and TFC) were obtained in seeds extract in the order of 50.51 mg GAE/g DE, and 8.30 mg mg RE/g DE, respectively. Similarly, *Opuntia* fruits extract exhibited a higher potent antioxidant activity and the IC50 value reached 0.22 mg/ mL. The major polyphenolic compounds, determined by HPLC, were gallic acid, procatechic acid, hydroxybenzoic acid and quinic acid for fruits extract. While sinapic acid and luteolin were the most compounds for seeds extract. These findings suggest that *O. dillenii* fruits and seeds extract are a promising natural source of bioactive molecules with beneficial properties for human health and could be useful in foods, cosmetics and pharmaceuticals industries.

***Corresponding author**
Kheiria Hcini, Biodiversity, Biotechnology and Climate Change Laboratory (LR11ES09), Department of Life Sciences, Faculty of Science of Tunis, Tunis El Manar University, 2092, Tunisia.

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Introduction

Aromatic and medicinal plants have been used for thousands of years as natural medicines, due to their richness in bioactive compounds with antioxidant potential and other beneficial properties [1,2]. In fact, these plants remain an essential vital source of polyphenolic components that have pharmacological properties, widely used in traditional and modern medicine, as well as for uses in phytotherapy and other industrial sectors [3]. Indeed, phenolic compounds have been studied for their biological properties with beneficial properties for human health and could be useful to replace or even decrease synthetic antioxidants in foods, cosmetics and pharmaceuticals industries [4]. Among the sources of these bioactive molecules, the *Opuntia* genus is particularly noted for its long history of use for different food, pharmaceutical and medicinal purposes, which could be attributed to its phytochemical constituents [5,6].

Opuntia spp. belongs to the Cactaceae family, native to the American continent, comprises more than 300 species and grows in very adverse conditions, making it especially interesting for cultivation in arid regions around the World [7]. Among those, *Opuntia dillenii (Ker Gawl)* Haw., known as the prickly pear, is of great interest for its richness in bioactive compounds and for its various therapeutic activities, including antioxidant, antimicrobial and anti-inflammatory activity [8-10]. It is commonly cultivated for its fruits, used as food, natural dye, sweetener and fodder, and is also recognized for its medicinal properties, including as an antidiabetic in traditional medicine [10,11,12]. Indeed, the seeds of *O. dillenii* may contribute to the higher antioxidant activity due to the high concentrations of polyphenols and flavonoids and the presence of a large amount of unsaturated fatty acids that provide valuable natural antioxidants for the pharmaceutical and food industry [13,14]. Due to their high content of phenolic compounds, which possess antioxidant, anticancer and neuroprotective properties, the *O. dillenii* L. fruits, particularly used for the treatment of gastritis, atherosclerosis and diabetes, are of great interest for potential applications in human health and medicine [15,16].

Therefore, it is important to make efforts to develop cactus production and increase its use in various fields. Thus, the evaluation of the phytochemical composition of fruits and seeds of *O. dillenii*. is of major interest. In this context, our study has been undertaken with the aim to estimate the total polyphenolic and flavonoids contents, to evaluate the antioxidant activity and to determine the polyphenolic profile of Tunisian cultivated *Opuntia* *dillenii* (Ker Gawl) Haw. fruits and seeds hydroethanolic extract. These finding allow the selection of this plant as a source of bioactive molecules with antioxidant potential and confirm them to be useful in the pharmaceuti¬cal, cosmetic, and food industries, with beneficial effects on human health.

Material and Methods

Total Polyphenol and Flavonoid Contents

The plant material used consists of mature fruits of Opuntia dillenii (Ker Gawl.) Haw., which were collected in January 2024, in the Gafsa region (southwest of Tunisia, Lower Arid, Latitude: 34°28'1.2"N, Longitude: 9°16'1.2"E). Voucher specimens of prickly pear (ODG25) was identified by Dr. Sondes Stambouli-Essassi and deposited at the herbarium of the Faculty of Sciences of Gafsa. After collection, the fruits were transported to the laboratory and were washed with tap water to remove dust and thorns. These fruits were divided into two batches, the first consists of whole fruits, which were cut and dried for 15 days. In the second batch, the fruits were peeled and the seeds were then separated from the pulp using distilled water, then rinsed and dried at room temperature away from sunlight, for 15 days. The two samples were then dried in an oven at 37 ° C for 48 h, until reaching a constant weight. After drying, fruits and seeds and peels were ground, using a coffee grinder, to obtain a fine powder and stored in dark glass bottles at 4 ° C until analysis.

Preparation of *Opuntia dillenii* (Ker Gawl) Haw. Extracts

Dried samples (2g) were macerated in 20 ml of hydroethanolic solvent (ethanol 75%) for 24 hours at room temperature [17]. The *Opuntia* fruits and seeds extract was filtered and dried in an oven at 37°C. The residue was redissolved in hydroethanol solvent and made up to 10 mL [18]. The yield of the extracts was expressed in terms of milligrams of dry hydroethanolic extract per gram of dry plant weight (mg DE/g DPW). The final extract was kept in vials at 4°C until the corresponding analyses were conducted.

Total Polyphenolic and Favonoid Contents

Total polyphenolic content (TPC) of *O. dillenii* hydroethanolic extracts was determined by the Folin-Ciocalteu reagent method [19]. A reaction mixture of 20 µL of the extract, 1155 µL of distilled water and 100 µL of 10% Folin-Ciocalteu reagent were prepared. A vigorous stirring was performed and 225 µL of a sodium carbonate (10%) were added. After 30 min of incubation at 25 °C, the absorbance of the resulting blue-colored solution was measured at 765 nm. Standard curve was prepared by using different concentrations ranging from 0.01 to 0.1 mg/mL of gallic acid. TPC was expressed as mg gallic acid equivalents per gram of dry extract (mg GAE/ g DE). Analyses were done in triplicate.

The determination of flavonoid content (TFC) was carried out by a spectrophotometric method with the aluminum trichloride ($AlCl_3$) reagent [20]. For each sample: 200 µL of hydroethanolic extract of *O. dillenii*, 1000 µL of distilled water and 50 µL of $NaNO_2$ (5%) were mixed. After 6 min, 120 µL of $AlCl_3$ (10%) was added and incubated for 5 min, followed by the addition of 400 µL of NaOH (1 M) and 230 µL of distilled water. The absorbance was measured at 510 nm by spectrophotometer against a blank tube without extract taken as a reference. Standard curve was prepared by using different concentrations of rutin (0.01; 0.02; 0.04; 0.06; 0.08 and 0.1 mg/mL). The TFC is expressed in mg of rutin equivalent/g of dry extract (mg RE/g DE). All experiments were carried out in triplicate.

DPPH• Radical-Scavenging Activity

The scavenging activity of the hydroethanolic *Opuntia* extracts was measured according to the method described by Brand-Williams et al., [21]. 500 µL of different extract, at different concentrations were added to 1000 µL of DPPH• solution (0.1 mM). Estimated time of reaction (30 min) was determined considering the reduction of the absorbance at 517 nm.The absorbance was measured at room temperature and darkness, against a blank (500 µL of sample plus 1000 µL of ethanol). All the assays were conducted in triplicate. The percent activity for the DPPH• technique was calculated according to:

$$\%I = [(Abs_{control} - Abs_{sample}) / Abs_{control}] \times 100$$

The results were expressed as the inhibitory concentration of the extract needed to decrease DPPH• absorbance by 50% (IC50). Concentrations are expressed in milligrams of dry extract per milliliter of hydroethanol (IC50, mg/mL).

Identification of Polyphenolic Compounds by HPLC-DAD Analysis

Polyphenolic compounds in *Opuntia dillenii* (Ker Gawl.) Haw. fruits and seeds hydoethanolic extract were identified and quantified by HPLC analysis based on the method adapted from [1]. Chromatographic analyses were performed on a reverse phase high-performance liquid chromatography (RP-HPLC) system using an Agilent1260 Series HPLC (Agilent Technologies, Germany) coupled to a diode array detector (HPLC-DAD). A 4.6 mm x 100 mm, 3.5 µm Hypersil ODS C18 reversed-phase column was used to separate individual phenolic compounds, at ambient temperature. The mobile phase was methanol (A) and acidified water containing 0.1% formic acid (B). The gradient program was as follows: 35% A/65% B, 0–6 min; 60% A/40% B, 6–9 min; 80% A/20% B, 9–14 min; 100% A/0% B, 14–25 min and 35% A/65% B, 25–30 min. The injection volume was 2 µL, the flow rate was 0.4 mL/min and the wavelengths of detection were set at 280 and 340 nm. The identification of the polyphenolic compounds was made through the comparison of retention times and spectra with those of commercially available standard compounds. For quantification, linear regression models were determined using standard dilution techniques.

Statistical Analysis

All experiments were performed in triplicate (n = 3) and data were reported as means ± standard deviation (SD). A General Linear Model procedure was carried out to assess for significant differences (significant model was accepted for a p-value < 0.05) using the IBM SPSS Statistic Program (v. 20).

Results

Total Polyphenol and Flavonoid Contents

The total polyphenol and flavonoid contents (TPC and TFC) of *O. dillenii* fruits and seeds hydroethanolic extract obtained by maceration are presented in Table 1. Highest TPC and TFC were obtained with seeds extract (50.51 ± 3.33 mg GAE/g DE and 8.30 ± 2.68 mg RE/g DE, respectively). These values prove that these extracts are rich in polyphenolic compounds. Our results are in agreement with those of, El Hassania et al., who also observed that the seeds extract had the highest content of total polyphenols (341.12 ± 78.90 mg GAE/100 g DM) [10]. On the other hand, the highest flavonoid concentration was measured in the juice extract of *O. dillenii* fruits (11.93 ± 11.72 mg QE/100 g DM), while the lowest flavonoid concentration was observed in the extracts of the bark and seeds (6.57 ± 1.32 and 6.63 ± 2.50 mg QE/100 g DM, respectively). In comparison with a previous study, *O. dillenii* plants growing in various Tunisian habitats revealed higher total phenolic content [9]. According to, Zourgui et al., for fruit extracts, the concentrations of total phenolic compounds were 24.65 mg EAG/g for ethanolic extracts and 12.78 mg EAG/g for aqueous extracts [22]. Regarding flavonoids, the observed values were 14.08 mg EAG/g for ethanolic extracts and 8.95 mg EAG /g for aqueous extracts.

This difference observed in the different studies can be explained by several factors, mainly, the low specificity of the Folin-Ciocalteu reagent, the extraction solvents which carries away non-phenolic substances such as sugars and proteins. Also, the distribution of secondary metabolites such as polyphenols can change depending on climatic conditions, plant maturity, storage conditions, harvest period and geographical location [12,23,24]. Polyphenols are mainly accountable for the antioxidant potential of aromatic and medicinal plants. The total phenolic content can be regarded as an important indicator of the antioxidant properties of plant extracts.

Table 1: Total Polyphenolic and Flavonïd Contents of Opuntia Fruits and Seeds Hydroethanolic Extract

	Total Polyphenolic Content (TPC, mg GAE/g DE)	Total Flavonoids Content (TFC, mg RE/g DE)	DPPH (IC_{50}, mg/mL)
Fruits extract (FE)	9.31 ± 0,33	1.65 ± 0.45	5.36 ± 0.27
Seeds extract (SE)	50.51 ± 3.33	8.30 ± 2.68	0.22 ± 0.04

DPPH Radical Scavenging Test

The ability to scavenge the DPPH free radical reached the value of 5.36 ad 0.22 milligrams of dry fruits and seeds hydroethanolic extract per milliliter of ethanol (mg/ mL), respectively (Table 1). The seeds extract proves a strong antioxidant activity. This result shows that plants with high antioxidant capacity are characterized by high levels of total phenolic content. The result of work carried out by Ben Lataief et al., showed that *O. dillenii* cladodes have a high antioxidant potential [9]. Furthermore, the anti-radical activity of *O. dillenni* seeds obtained by Bouhrim et al., showed that the ethanolic extracts of *O. dillenni* seeds have a high antioxidant power with an IC_{50} of the order of 0.38± 0.08 mg/ml, which are closer to our results [25]. Several authors have published the important role of polyphenolic compounds on the antioxidant power of the *Opuntia* seeds extract [26].

In fact, polyphenolic extracts derived from various part of plant exhibit significant antioxidant properties, playing a crucial role in mitigating oxidative stress-related diseases. Due to their potent bioactive compounds, these extracts are increasingly utilized as natural preservatives and functional ingredients in food products [6,27]. Therefore, it can be noted that there is a significant positive correlation between the concentration of polyphenols and the antioxidant activity, which confirms that polyphenols are powerful antioxidants capable of inhibiting the formation of free radicals and opposing the oxidation of macromolecules [5]. It can be confirmed that the concentrations of polyphenolic compounds have a significant role in the antioxidative power of the plant extract. The polyphenols appear to be effective donors of hydrogen to the DPPH radical because of their ideal structural chemistry.

Polyphenolic Profile of *O. dillenii* Hydroethanolic Extract

HPLC analysis revealed the presence of fifteen polyphenolic compounds in *Opuntia dillenii* fruits and seeds hydroethanolic extract (Table 2 and figure 1). Among the mentioned phenolic compounds, gallic acid and sinapic acid were found to be the most abundant compounds. The major polyphenolic compounds, determined by HPLC, were gallic acid, procatechic acid, 4-hydroxybenzoic acid and quinic acid for fruits extract (2699.03, 340.72, 262.79, and 260.34 %, respectively). While sinapic acid luteolin, and catechin were the most compounds for seeds extract (737.87, 102.13, and 98.22%, respectively). Previous studies conducted by El Hassania et al., showed that gallic acid, vanillic acid, and syringic acid were the major constituents detected in the *O. delleniii* extract from populations located at different geographic origins in Morroco [10]. It is well known that most of these molecules display antioxidant and antimicrobial activities and those they can prevent chronic diseases such as cardiovascular disease, inflammation, and cancer [28,29].

Table 2: Polyphenolic Compounds Identified in O. dellenii Fruits and Seeds. Hydroethanolic Extract

N°	Polyphenolic compounds	RT	Fruits extract (%)	RT	Seeds extract (%)
1	Quinic acid	3.69	260.34	3.63	36.21
2	4-hydroxybenzoic acid	4.13	262.79	4.28	55.44
3	Protocatchuic acid	4.28	340.72	-	nd
4	Vanillic acid	5.01	90.91	5.01	30.92
5	Vanillin	6.10	30.43	-	nd
6	Gallic acid	7.67	2699.03	-	nd
7	Catechin	-	nd	10.04	98.22
8	Caffeic acid	-	nd	-	nd
9	syringic acid	-	nd	13.18	42.69
10	Rutin	-	nd	-	nd
11	Sinapic acid	17.68	79.55	17.67	737.87
12	Ferulic acid	18.47	76.62	-	nd
13	Trans-3-hydroxycinnamic acid	-	nd	19.43	79.03
14	Myricetin	-	nd	21.93	55.15
15	Luteolin	-	nd	24.85	106.13

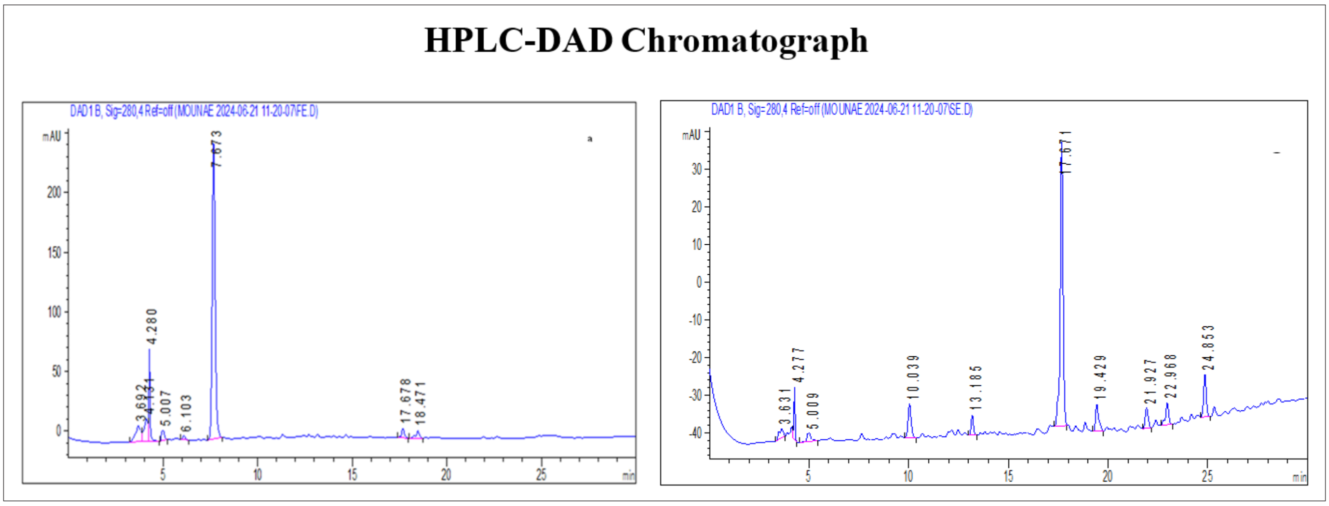

Figure 1: HPLC Chromatogram of *Opuntia dillenii* Fruits (a) and Seeds (b) Hydroethanolic Extract Extract at λ = 280 nm

The present research highlighted that O. dillenii constitute a great source of flavonoids and polyphenols, which are important plant secondary metabolites. The biological properties of O. dillenii are the subject of numerous studies, due to the numerous bioactive molecules derived from its various parts. Indeed, O. dillenii flowers have anti-inflammatory, analgesic, antioxidant, antiviral, and antimicrobial effects [13]. Furthermore, O. dillenii seed oil possesses antidiabetic and antioxidant activities correlated with its phenolic compound content [25]. Indeed, previous studies revealed significant correlations between various phenolic compounds and the antioxidant activity, proving the significance of these compounds and their contribution to the antioxidant power of the plant extract [5,28,30,31]. The interaction or synergistic effect among the polyphenolic compounds contained in sage extract may also contribute to their antioxidant capacity.

Conclusion

The present study has investigated the variation in total polyphenolic content (TPC), total flavonoïds content (TFC) and antioxidant activity of Opuntia dillenii fruits and seeds hydrethanolic extract. This highlights confirmed that Opuntia fruits and seeds extract have proven to be an effective potential source of polyphenols and proved that the plants with high levels of total polyphenolic content are characterized by high antioxidant capacity. The qualitative and quantitative identification performed by HPLC analysis allowed identifying and quantifying fifteen polyphenolic compounds. Among the mentioned phenolic compounds, gallic acid and sinapic acid were found to be the most abundant compounds. Opuntia fruits and seeds have proven to be an effective potential source of polyphenols and could be useful in replacing or even decreasing synthetic antioxidants in foods, cosmetics and pharmaceutical products. Moreover, although preliminary evidence suggests that Opuntia seeds polyphenolic compounds may possess promising beneficial effects, further in vitro and in vivo investigations (antidiabetic, antibacterial, and antibiofilm activities) are needed to fully understand its biological properties and potential therapeutic applications in humans [32].

Credit Authorship Contribution Statement

Farah Zidi: Methodology, Investigation, Formal analysis. Amel Azaza: Methodology, Formal analysis. Monia Bendhifi-Zaroug: Methodology, Formal analysis. Abdallah Fraj: Methodology, Formal analysis, Mouna Ben Farhat: Methodology, Formal analysis, Kheiria Hcini: Conceptualization, Methodology, Investigation, Writing-original draft, Writing-Review & Editing, Resources, Visualization, Validation, Supervision, project administration. All authors have read and agreed to the published version of the manuscript.

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Declaration of Competing Interest: The authors declare that they have no known conflict of interest or personal relationships that could have appeared to influence the work reported in this paper.

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