Research Article

Chemical Composition, Antibacterial, Antifungal and Antidiabetic Activities of Ethanolic Extracts of *Opuntia dillenii* Fruits Collected from Morocco

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*Opuntia dillenii* (Ker Gawl.) Haw. belongs to the *Cactaceae* family and is native to the arid and semi-arid regions of Mexico and the southern United States. *O. dillenii* are now used as medicinal plants in various countries. In this study, we investigated the chemical composition of ethanolic extracts obtained from seeds, juice, and peel of *O. dillenii* fruits collected from Morocco, and we evaluated their antibacterial, antifungal, and antidiabetic activities. Phytochemical screening revealed high quantities of polyphenols (193.73 ± 81.44 to 341.12 ± 78.90 gallic acid eq [g/100 g dry weight]) in the extracts. The major phenolic compounds determined by HPLC were gallic acid, vanillic acid, and syringic acid. Regarding flavonoids, quercetin 3-O-β-D-glucoside and kaempferol were the predominant molecules. Juice extracts showed weak to moderate antibacterial activity against the bacteria species *Listeria monocytogenes*, *Escherichia coli*, and *Salmonella braenderup*. All tested extracts displayed a significant inhibitory effect on α-glucosidase and α-amylase activities *in vitro*, with the peel extracts showing the greatest inhibitory effects. Together, these findings suggest that *O. dillenii* fruits are a promising source for the isolation of novel compounds with antibacterial or antidiabetic activities. For the most abundant phytochemicals identified in *O. dillenii* peel ethanolic extract, molecular docking simulations against human pancreatic α-amylase enzyme were performed. These indicated the presence of bioactive compounds in the extract with a better potential to decrease the enzyme activity than the commercial drug acarbose.
1. Introduction

*Opuntia* spp. are plants belonging to the *Cactaceae* family [1, 2] which are native to arid and semi-arid regions of Mexico and the southern United States [3, 4] and were introduced to Europe and North Africa in the 16th century [5, 6]. Among those, *Opuntia dillenii* (Ker Gawl.) Haw. is one of the most important cacti for agronomic and medicinal uses in many countries, as it represents a potential source of various chemical compounds of biological and pharmacological importance. Plant secondary metabolites display recognized therapeutic benefits. As plant materials have long been used as traditional medicines for healing without knowing the exact origin of their actions, the characterization of their secondary metabolites has been the subject of numerous research based on *in vitro* and *in vivo* studies. This is particularly the case for phenolic compounds, including polyphenols and flavonoids, which are the subject of the present study. These compounds are widely used in therapy due to their anti-inflammatory, enzyme inhibitory, antioxidant, and antidiarical activities [7–9]. They are also used as additives in the food, pharmaceutical, and cosmetic industries [10].

Many researchers have focused their investigations on characterizing the properties of plants of the genus *Opuntia* to form a useful database for the prevention and treatment of chronic diseases [11, 12]. In particular, plants of this genus are known for their analgesic, anti-hyperglycemic [13], inflammatory [14], antifungal [15], antitumor [16, 17], hepatoprotective [18, 19], and antidiabetic activities [16, 20, 21]. *Opuntia* fruits are a rich source of dietary fiber, vitamins (B1, B2 and C) and natural colorings, i.e. betanin and indicaxanthin [22–27], which are responsible for the intense red coloring of the fruit [27], which are used to prevent certain diseases. *Opuntia dillenii* fruits are notably used for the treatment of gastritis, atherosclerosis and diabetes due to their high content of phenolic compounds, which provide antioxidant, anticancer and neuroprotective properties [9] and are of great interest for potential applications in human health and medicine. As efforts are underway in Morocco to develop cactus production and to increase its use in various common foods, the evaluation of the chemical composition of the pulp, peel, and seeds of edible *Opuntia* is of high interest.

Previous studies have described the chemical composition of *O. dillenii* fruits. However, no study has yet compared the chemical composition of different parts of the fruits as well as the effect of the content of these compounds on the associated biological activities. The present work aimed to compare the chemical compositions of different extracts of seeds, juice, and peel of *O. dillenii* fruits harvested from Morocco. Moreover, the biological activities of those extracts were evaluated. More specifically, the antimicrobial (antifungal and antibacterial) properties, as well as the pancreatic α-amylase and intestinal α-glucosidase inhibitory activities of the ethanolic extracts, were determined.

2. Experimental Section

2.1. Chemicals and Reagents. n-hexane, ethyl acetate, and ethanol were purchased from Merck (Darmstadt, Germany). All chemicals and reagents used for the determination of total phenolic compounds were of analytical grade and were purchased from Merck and Carl Roth GmbH (Karlsruhe, Germany). 3,5-Dinitrosalicylic acid (DNSA), acarbose, α-glucosidase, and α-amylase were purchased from Merck (Sigma-Aldrich, St. Louis, MA, USA). The kit necessary for the measurement of blood sugar was purchased from Bio-systems (Barcelona, Spain). The bacterial strains were supplied by the Pasteur Institute of Casablanca, Morocco. The fungal strains were provided by Professor Adnane Sellam (Université Laval, Canada).

Phenolic standards: gallic acid, vanillic acid, syringic acid, salicylic acid, 4-hydroxybenzoic acid, vanillin, ferulic acid, caffeic acid, p-coumaric acid, cinnamic acid, sinapic acid, quercetin, kaempferol, rutin, quercetin 3-O-β-D-glucoside, catechin, naringin, and chalcone were purchased from Merck and Carl Roth GmbH (Karlsruhe, Germany).

2.2. Sample Collection and Preparation. Mature purple fruits of *O. dillenii*, the prickly pear plant, were collected in February 2017 in Morocco. The plant species was authenticated by Mohammed Fennan, a botanical expert from the Scientific Institute of Mohammed V University. The specimen was deposited at Mohammed First University, Oujda, Morocco, under the reference number HUMPOM 351 [2].

The collected fruit specimens were washed with water to remove dust and spines, peeled and blended (Facilic glass LM310E; Groupe Seb, Mayerne, France), then the seeds were separated from the juice by passing the mixture through a sieve with a 2 mm mesh size (Figure 1). The juice was dehydrated by heating at 40°C in an oven for 2 days. Seeds and peels were washed with distilled water, dried at 20°C for 1 week, weighed, and then reduced to a fine powder using a Moulinex coffee grinder (DPA241; Groupe Seb Lourdes, France). All components (seeds, juice, and peel) were stored at −20°C for a maximum of eight weeks.

2.3. Preparation of Opuntia dillenii Fruit Extracts. One hundred grams of the ground seeds, juice, and peel of *O. dillenii* fruits were macerated with 200 mL of n-hexane to remove fatty acids. The mixtures were stirred at 20°C for 24 h and filtered through a glass filter crucible (50 mL, Porosity 4; Isolab Wertheim, Germany). The extracts were then concentrated using a rotary evaporator (Laborota 4000; Heidolph Instruments, Schwabach, Germany) under reduced pressure at 40°C to obtain the corresponding powder. Then, ethyl acetate and ethanol were used successively to obtain the corresponding extracts by repeating the maceration process (Figure 2). The extracts were stored in dark bottles at 4°C until use.

2.4. Total Polyphenol Content. The total phenol concentration in the *O. dillenii* ethanolic extracts was determined...
using the Folin-Ciocalteu method with some modifications. A standard curve was first plotted using gallic acid as a standard. To prepare the calibration curves and samples, 200 \( \mu \)L of ethanolic gallic acid solutions (0.78, 1.56, 3.12, 6.25, 12.5, 25, 50 and 100 \( \mu \)g/mL) or 200 \( \mu \)L of \( O. \) dillenii ethanolic extract were mixed up with 1000 \( \mu \)L of the Folin-Ciocalteu reagent and 800 \( \mu \)L (75g/L) of sodium carbonate (Na\(_2\)CO\(_3\)). The mixture was incubated for 1h, and the absorbance was measured at 765 nm against ethanol as a blank. All samples were analyzed in triplicate. Data obtained are the average of one analysis performed in triplicate. Data for the total phenol concentration were expressed as mg gallic acid equivalent (GAE)/100 g of dry matter of the extract.

2.5. Total Flavonoid Content. Total flavonoids were measured according to the method described by Kim et al. with some modifications [28]. In brief, 200 \( \mu \)L of \( O. \) dillenii ethanolic extract, 1000 \( \mu \)L of distilled water and 50 \( \mu \)L of NaNO\(_2\) (5% W/V) were mixed up. After 6 min, 120 \( \mu \)L of AlCl\(_3\) (10% W/V) was added and incubated for 5 min, followed by the addition of 400 \( \mu \)L of NaOH (1 M) and 230 \( \mu \)L of distilled water. The calibration curve was constructed using quercetin standard solutions at different concentrations (0.78, 1.56, 3.12, 6.25, 12.5, 25, 50 and 100 \( \mu \)g/mL). The absorbance was measured at 510 nm using ethanol as a blank. All measures were performed in triplicate. Data for the total phenol concentration were expressed as quercetin equivalents (QE) per milligram of dry matter of the extracted sample.

2.6. Quantification of Phenolic Compounds in \( O. \) dillenii Ethanolic Extracts by HPLC. The phenolic compounds in the ethanolic extracts were determined by HPLC using an Agilent 1200 (Agilent Technologies, Palo Alto, CA, USA) connected to a diode array UV detector (Bruker, Germany). Each extract (20 \( \mu \)L) was injected into a Zorbax XDB-C18 (5 \( \mu \)m porosity, 250 \( \times \) 4.6 mm; Agilent Technologies series 1100 system (Palo Alto, CA, USA)) column equipped with a 4 \( \times \) 3 mm C18 cartridge precolumn (Agilent Technologies) with the following elution gradient: 0–25 min, 20% B; 25–30 min, 100% B and 30–35 min, 20% B. The mobile phases used for the elution of samples were A (water/0.3% phosphoric acid) and B (methanol), with a flow rate of 1 mL/min. Separation was performed at a constant temperature of 40°C. Spectrophotometric detection was performed at 254, 280, 320, 350, and 540 nm. Compounds were identified by comparison of their retention times and UV spectra with those of authentic standards. The quantification (Table 1) was performed using calibration curves with different concentrations of standards in ethanol (0.2; 0.4; 0.6; 0.8; 1 mg/mL). For each molecule, the limit of detection (LOD) and limit of quantification (LOQ) [29] were determined using the following formulas:

\[
LOD = 3.3 \left( \frac{\text{standard deviation of signal intensity from low – concentration samples}}{\text{slope of the calibration curve}} \right),
\]

\[
LOQ = 10 \left( \frac{\text{standard deviation of signal intensity from low – concentration samples}}{\text{slope of the calibration curve}} \right).
\]
Each calibration curve was repeated three times. Results were expressed as mg/100 g of dry matter. Analyses were performed in triplicate.

2.7. Antifungal Activity. The antifungal activity of the extracts was measured using liquid cell culture assays as described in the protocol by Bay et al. [30]. Four fungal species were used: *Saccharomyces cerevisiae* (BY4741), *Candida albicans* (SC5314), *Candida krusei* (ATCC6258), and *Candida tropicalis* (My070362). The growth rate of the yeast cells in the presence or absence of plant extracts was monitored by measuring the optical density at 600 nm (OD600) using a V-1200 spectrophotometer (Shanghai Mapada Instruments CO. LTD, Shanghai, China) as described by Bouchal et al. [31]. All experiments were repeated three times, with the residual extract of *Fredolia aretioides* used as a positive control (control +) according to Bouchal et al. [32].

2.8. Antibacterial Activity. Antibacterial activity was evaluated using a disc diffusion test as previously reported by Abrigach et al. [33]. Two Gram-positive bacteria species (*Staphylococcus aureus* and *Listeria monocytogenes*) and two Gram-negative bacteria species (*Escherichia coli* and *Salmonella braenderup*) were used in this study. Cells were first spread on Petri plates containing solid LB medium. Then, paper discs (6 mm in diameter) that had been impregnated with the tested compounds were placed on the inoculated agar plates and incubated at 37°C. All the extracts were used at a concentration of one milligram per disc. Twenty-four hours later, the antibacterial activity was assessed by measuring the diameter of the growth-inhibition zone in millimeters. The inhibition zones were measured three times for each extract, and the antibiotic streptomycin was used as a positive control.

The minimum inhibitory concentration (MIC) was defined as the lowest concentration that inhibits bacterial growth following incubation for 24 h. The MIC was determined by visual observation of the red color indicator [34, 35]. All experiments were carried out in triplicate, and means were calculated.

2.9. In Vitro α-Amylase Inhibition Assay. The inhibition of pancreatic α-amylase activity by *O. dilleni* extracts was studied using the procedure described by Daoudi et al. with some modifications [36, 37]. The assay mixtures contained 200 μL of pancreatic α-amylase enzyme solution (13 IU), 200 μL of phosphate buffer (0.02 M; pH 6.9) and 200 μL of *O. dilleni* extracts (0.5 or 1 mg/mL) or acarbose (0.5 or 1 mg/mL). Acarbose is a specific inhibitor of intestinal alpha-glucosidase and pancreatic alpha-amylase enzymes [38]. The mixtures were pre-incubated at 37°C for 10 min. Then, 200 μL of starch (1% W/V) dissolved in phosphate buffer was added to each tube and incubated for 20 min at 37°C. To stop the enzymatic reaction, 600 μL of DNSA color reagent was added. Immediately afterwards, the tubes were incubated for 8 min at 100°C, then they were placed in an ice-cold water bath for a few minutes. The mixture was diluted by adding 1 mL of distilled water, and the absorbance was measured at 540 nm. The inhibition percentage was calculated using the following formula:

$$\alpha – \text{amylase inhibition percentage} = \left( \frac{[\text{OD test} 540 \text{ nm} - \text{OD control} 540 \text{ nm}]}{\text{OD test} 540 \text{ nm}} \right) \times 100. \quad (3)$$

### Table 1: Linearity of phenolic compounds standards and sensitivity of the HPLC-DAD method.

| N° | Compounds                  | Concentration range (mg/mL) | Linear regression $y = ax + b$ | $r^2$ | LODb (mg/mL) | LOQc (mg/mL) |
|----|----------------------------|-----------------------------|-------------------------------|------|--------------|--------------|
| 1  | Gallic acid                | 0.2–1                       | $y = 904.2x + 40.6$           | 0.99 | 0.02         | 0.05         |
| 2  | Catechin                   | 0.2–1                       | $y = 2784.3x + 133.3$         | 0.99 | 0.05         | 0.16         |
| 3  | 4-Hydroxybenzoic acid      | 0.2–1                       | $y = 3406.7x + 235.1$         | 0.99 | 0.01         | 0.03         |
| 4  | Vanillic acid              | 0.2–1                       | $y = 2040.9x + 124.8$         | 0.99 | 0.03         | 0.09         |
| 5  | Caffeic acid               | 0.2–1                       | $y = 24178x + 522.4$          | 0.99 | 0.01         | 0.02         |
| 6  | Syringic acid              | 0.2–1                       | $y = 14815x + 177.8$          | 0.99 | 0.01         | 0.03         |
| 7  | Vanillin                   | 0.2–1                       | $y = 1622x + 91.9$            | 0.99 | 0.02         | 0.07         |
| 8  | p-Coumaric acid            | 0.2–1                       | $y = 4265.3x + 40.4$          | 0.99 | 0.02         | 0.05         |
| 9  | Sinapic acid               | 0.2–1                       | $y = 1052.4x + 34.2$          | 0.99 | 0.03         | 0.09         |
| 10 | Ferulic acid               | 0.2–1                       | $y = 987.9x + 3.5$            | 0.99 | 0.02         | 0.06         |
| 11 | Naringin                   | 0.2–1                       | $y = 443.3x + 15.3$           | 0.99 | 0.01         | 0.03         |
| 12 | Rutin                      | 0.2–1                       | $y = 578.4x + 8.3$            | 0.99 | 0.01         | 0.04         |
| 13 | Salicylic acid             | 0.2–1                       | $y = 815.1x + 12.2$           | 0.99 | 0.03         | 0.08         |
| 14 | Quercetin                  | 0.2–1                       | $y = 7989.6x + 116.2$         | 0.99 | 0.02         | 0.05         |
| 15 | Cinnamic acid              | 0.2–1                       | $y = 828.1x - 18.1$           | 0.99 | 0.05         | 0.16         |
| 16 | Quercetin 3-O-β-D-glucoside| 0.2–1                       | $y = 1090.6x + 15.2$          | 0.99 | 0.02         | 0.07         |
| 17 | Kaempferol                 | 0.2–1                       | $y = 1000.9x + 12.5$          | 0.99 | 0.03         | 0.09         |
| 18 | Chalcone                   | 0.2–1                       | $y = 2666x + 5.1$             | 0.99 | 0.01         | 0.04         |

*a*, Correlation coefficients of the regression equation. *b*, LOD limit of detection. *c*, LOQ limit of quantification.
Table 2: Yields of the n-hexane, ethyl acetate and ethanolic extracts of O. dillenii.

| Solvents     | Seeds            | Juice           | Peel            |
|--------------|------------------|-----------------|-----------------|
| N-hexane     | 8.41±4.52        | 0.52±0.14       | 1.33±0.38       |
| Ethyl acetate| 1.07±0.23        | 0.53±0.30       | 1.00±0.10       |
| Ethanol      | 0.66±0.17        | 20.30±0.58      | 10.11±0.29      |

2.10. In Vitro α-Glucosidase Inhibition Assay. The effects of the O. dillenii extracts on the intestinal α-glucosidase activity were quantified by monitoring the glucose released from sucrose degradation according to the protocol described by Ouassou et al. with some modifications [39, 40]. The assay mixtures contained 100 μL of sucrose (50 mM), 1000 μL of phosphate buffer (50 mM; pH 7.5) and 100 μL of intestinal α-glucosidase enzyme solution (10 IU). Then, 10 μL of distilled water (control), acarbose (positive control) or O. dillenii extract solutions at two different concentrations (166 or 328 μg/mL) were added to the mixture, which was incubated at 37°C in a water bath for 25 min. The mixture was then heated at 100°C for 5 min to stop the enzymatic reaction, and the release of glucose was estimated by the glucose oxidase method using a commercial auto-kit (GLUCOSE, Biosystems). The absorbance was measured at 500 nm, and the inhibition percentage was calculated using the following formula:

\[
\alpha - \text{glucosidase inhibition percentage} = \left( \frac{\text{OD control 500 nm} - \text{OD test 500 nm}}{\text{OD control 500 nm}} \right) \times 100.
\]

2.11. Molecular Docking Study. The structures of major compounds present in O. dillenii peel ethanolic extract, which were chosen as the representatives of different metabolites’ classes (phenolic acids, phenolic alcohols, flavonols, flavanols, flavanones, and chalcones), were downloaded from PubChem database (https://pubchem.ncbi.nlm.nih.gov/). The compounds were gallic acid (CID: 370), p-coumaric acid (CID: 637542), kaempferol (CID: 5280863), catechin (CID: 90644), naringin (CID: 442428), chalcone (CID: 637760). Acarbose (CID: 41774) was used as the standard drug. X-ray crystal structure of human pancreatic α-amylase (pdb code: 2qv4) [41] enzyme used in this study was retrieved from the RCSB Protein Data Bank (https://www.rcsb.org/). The structure of the receptor was prepared before docking by eliminating unnecessary molecules (water molecules, inhibitors, cofactors). Missing polar hydrogen atoms were also added with the aid of BIOVIA Discovery Studio Visualizer 2020 PyRx 8.0 software [42] was utilized to perform the molecular simulations. The dimensions of the grid box were defined to enclose the active pocket of protein. The grid box was 30×30×30 grid points in size centered at 12.20×50.06×26.87. The binding affinity of compounds toward the receptor was evaluated based on Gibbs’s free energy values calculated by the docking process. Analysis and visualization of the results were carried out using the BIOVIA Discovery Studio Visualizer 2020.

3. Statistical Analysis

All data are presented as the mean ± SD. Multiple comparisons of mean values were set up using one-way parametric analysis of variance (ANOVA). The degree of statistical significance of the data was considered with a probability of (p < 0.05) using STATISTICA software (version 7.1).

4. Results

4.1. Yields of the Ethanolic Extracts. The yields of the n-hexane, ethyl acetate and ethanolic extracts of O. dillenii seeds, juice and peel obtained by maceration in the present study are presented in Table 2. The highest yields were obtained with ethanol extracts (8.41±5.19%). The n-hexane extract of seeds was more abundant in fatty acids than those that were from the juice and peel (8.41±5.19%; 0.52±0.14% and 1.33±0.38%, respectively). In addition, the lowest yields were obtained with ethyl acetate extracts (between 0.53±0.30 and 1.07±0.23%). Regarding the ethanolic extracts, the highest yields were obtained with the juice (20.30±0.58%) in comparison with the other parts of the fruits. Therefore, the extraction yield depended on the polarity of the solvent.

4.2. Phytochemical Analysis of the Ethanolic Extract

4.2.1. Total Polyphenol and Flavonoid Contents. According to the results obtained using the Folin-Ciocalteu method (Table 3), the total phenolic content of the ethanolic extracts obtained from O. dillenii harvested in Morocco ranged between 193.73±81.44 and 341.12±78.90 mg GAE/100 g DW. The seeds extract had the highest total phenolic content (341.12±78.90 mg GAE/100 g DW) while the juice extract and the peel extract had the lowest phenolic contents (193.73±81.44 and 240.91±99.70 mg GAE/100 g DW, respectively). Concerning the flavonoid analysis, results showed low flavonoid content in the extracts, as the highest flavonoid concentration measured in the juice extract of...
Table 3: Phytochemical analysis of ethanolic extracts of seeds, juice and peel of *O. dillenii* fruits.

| Samples     | Total polyphenol content (mg GAE/100 g DW) | Total flavonoid content (mg QE/100 g DW) |
|-------------|-------------------------------------------|-----------------------------------------|
| Seeds       | 341.12 ± 78.90                            | 6.63 ± 2.50                             |
| Juice       | 193.73 ± 81.44                            | 11.93 ± 11.72                           |
| Peel        | 240.91 ± 99.70                            | 6.57 ± 1.32                             |

Table 4: HPLC-DAD analysis of the four families of phenolic compounds; phenolic acids, phenolic alcohols, flavonoids and chalcones; which were detected in seeds, juice and peel of ethanolic extracts of *O. dillenii* fruits harvested in Morocco.

| Sample (mg/100g) | Retention time (Tr) (min) | Seeds | Juice | Peel |
|------------------|---------------------------|-------|-------|------|
| Gallic acid      | 2.40                      | 4.95 ± 1.87 | 447.63 ± 292.59 | 183.04 ± 215.78 |
| Syringic acid    | 6.72                      | 252.74 ± 139.85 | 63.07 ± 44.13 | 101.88 ± 73.78 |
| Salicylic acid   | 11.30                     | 29.79 ± 23.65 | 8.88 ± 2.26 | 117.53 ± 73.88 |
| 4-Hydroxybenzoic acid | 5.89                  | 40.54 ± 20.74 | 59.43 ± 42.24 | 26.46 ± 29.13 |
| Ferulic acid     | 8.15                      | 19.46 ± 13.29 | 13.40 ± 6.31 | 18.55 ± 3.45 |
| Caffeic acid     | 6.40                      | 15.14 ± 9.41 | 34.63 ± 50.49 | 71.13 ± 8.44 |
| p-Coumaric acid  | 8.15                      | 7.70 ± 11.98 | 13.10 ± 10.42 | 109.22 ± 78.73 |
| Cinnamic acid    | 12.40                     | 26.03 ± 12.59 | 10.64 ± 1.20 | 26.05 ± 14.88 |
| Sinapic acid     | 8.41                      | 11.96 ± 7.09 | 50.60 ± 23.64 | 62.59 ± 4.61 |
| Quercetin        | 12.21                     | 38.95 ± 20.63 | 16.27 ± 9.57 | 20.78 ± 4.85 |
| Kaempferol       | 13.72                     | 104.78 ± 94.64 | 94.90 ± 21.33 | 166.68 ± 54.85 |
| Rutin            | 9.64                      | 100.96 ± 54.92 | 18.10 ± 9.26 | 31.28 ± 3.39 |
| Quercetin 3-O-β-D-glucoside | 9.60          | 199.66 ± 212.15 | 57.80 ± 21.55 | 153.15 ± 8.09 |
| Catechin         | 4.07                      | 15.18 ± 14.42 | 113.67 ± 103.42 | 32.27 ± 18.07 |
| Naringin         | 9.39                      | 28.42 ± 16.19 | 20.69 ± 8.26 | 13.28 ± 7.49 |
| Chalcone         | 17.39                     | 35.30 ± 16.70 | 10.70 ± 1.10 | 18.73 ± 6.95 |

Figure 3: Example of a HPLC-DAD chromatogram obtained following analysis of *O. dillenii* ethanolic extract at 280 nm and of standards (Std; gallic acid 1, catechin 2, 4-hydroxybenzoic acid 3, vanillic acid 4, caffeic acid 5, syringic acid 6, vanillin 7, p-coumaric acid 8, sinapic acid 9, ferulic acid 10, naringin 11, rutin 12, salicylic acid 13, quercetin 14, cinnamic acid 15, quercetin 3-O-β-D-glucoside 16, kaempferol 17, trans-chalcon 18).

Table 5: Families of phenolic compounds in ethanolic extracts of *O. dillenii* seeds, juice and peel. Values are means of three determinations ± standard errors of the mean (SEM).

| Families of phenolic compounds | Seeds (mg/100g) | Juice (mg/100g) | Peel (mg/100g) |
|--------------------------------|-----------------|-----------------|----------------|
| Phenolic acids (%)             | 46.64 ± 11.26   | 60.07 ± 8.74    | 38.50 ± 14.17  |
| Phenolic alcohols (%)          | 5.77 ± 0.65     | 10.74 ± 3.68    | 24.55 ± 7.70   |
| Flavonoids (%)                 | 44.33 ± 10.51   | 28.03 ± 4.96    | 35.39 ± 6.44   |
| Chalcones (%)                  | 3.25 ± 1.01     | 1.18 ± 0.63     | 1.54 ± 0.36    |
O. dillenii fruits was 11.93 ± 11.72 mg QE/100 g DW. The lowest flavonoid concentration was measured in the peel and seed extracts (6.57 ± 1.32 and 6.63 ± 2.50 mg QE/100 g).

4.2.2. HPLC Analysis of O. dillenii Ethanolic Extracts. The chemical composition of O. dillenii fruit Ethanolic extracts (Table 4 and Figure 3) was determined by HPLC-DAD. Four families of phenolic compounds were detected in the extracts (Table 5). Phenolic acid derivatives were the most abundant molecules (38.50 ± 14.17–60.07 ± 8.74%), followed by flavonoids (28.03 ± 4.96–44.33 ± 10.51%), phenolic alcohols (5.77 ± 0.65–24.55 ± 7.70%) and chalcones (1.18 ± 0.63–3.25 ± 1.01%). Gallic acid, vanillic acid, and syringic acid were the major constituents detected in the extracts. Results also showed that the concentrations of different phenolic compounds depended on the extracted part of the O. dillenii fruit. In all extracts, the juice extract presented the highest gallic acid concentration (447.63 ± 292.59 mg/100 g) followed by the peel extract (183.04 ± 215.78 mg/100 g), while the gallic acid concentration found in seeds extract was the poorest (4.95 ± 1.87 mg/100 g). The seeds presented the highest amount of syringic acid (252.74 ± 139.85 mg/100 g) followed by the peel (101.88 ± 73.78 mg/100 g). Salicylic acid was more abundant in the peel, with a level of 117.53 ± 73.88 mg/100 g. Finally, O. dillenii fruit ethanolic extracts (peel, seeds and juice). Cells were cultured in the presence or absence of 1 mg/mL of each extract and the growth rate of cells was monitored every 2 h by measuring the OD_{600}, as described in the materials and methods section. The residual extract of Fredolia aretioides was used as a positive control due to its recognized antifungal activity [32] and DMSO was used as a negative control. All experiments were carried out in triplicate. Error bars are shown; when they are not visible, they are shown smaller than the data symbols.

**Figure 4:** Antifungal activity of O. dillenii fruit ethanolic extracts (peel, seeds and juice). Cellswere cultured in the presence or absence of 1 mg/mL of each extract and the growth rate of cells was monitored every 2 h by measuring the OD_{600}, as described in the materials and methods section. The residual extract of Fredolia aretioides was used as a positive control due to its recognized antifungal activity [32] and DMSO was used as a negative control. All experiments were carried out in triplicate. Error bars are shown; when they are not visible, they are shown smaller than the data symbols.

**Table 6:** Antibacterial activity of ethanolic extracts of O. dillenii on *Listeria monocytogenes*(a), *Staphylococcus aureus*(b), *Salmonella braenderup*, (c) and *Escherichia coli*(d).

| Extracts/Antibiotic | *Escherichia coli* | *Salmonella braenderup* | *Staphylococcus aureus* | *Listeria monocytogenes* |
|---------------------|-------------------|------------------------|------------------------|-------------------------|
| Seeds               | nd                | nd                     | nd                     | nd                      |
| Peel                | nd                | nd                     | nd                     | nd                      |
| Juice               | 7.33 mm           | 7.33 mm                | nd                     | 8.66 mm                 |
| Streptomycin        | 20 mm             | 18 mm                  | 14 mm                  | 11 mm                   |

*, Mean of three repetitions; nd, not detected.
the flavonoid profile, quercetin 3-O-β-D-glucoside was highly dominant in seeds extract (199.66 ± 212.15 mg/100 g), followed by peel extract (153.15 ± 8.09 mg/100 g). Kaempferol was also abundant in almost all the extracts, but the peel presented the highest amount (166.68 ± 54.85 mg/100 g). On the other hand, chalcone was present at low concentrations.

5. Antifungal and Antibacterial Activities of *O. dillenii* Ethanolic Extracts

A previous study already showed that *O. dillenii* fruit extracts have no antifungal activity against *Candida albicans*, which is the most common human fungal pathogen [17]. In the present work, we evaluated the antifungal effects of *O. dillenii* ethanolic fruit extracts on other *Candida* species involved in invasive fungal infections. These extracts were also evaluated for their antifungal activity against the budding yeast *Saccharomyces cerevisiae*, which is an excellent model system for studying antifungal compounds. As shown in Figure 4, all ethanolic extracts (peel, seeds, and juice) of *O. dillenii* harvested from Morocco showed no antifungal activity against any of the fungi species when used at 1 mg/mL.

The antibacterial activity of *O. dillenii* ethanolic fruit extracts was evaluated against four different bacterial strains, as described in the Materials and Methods section. As shown in Table 6, the ethanolic extracts from *O. dillenii* peel or seeds showed no activity against any of the tested strains. Similarly, the ethanolic juice extracts of *O. dillenii* showed no activity against *Staphylococcus aureus*. Interestingly, ethanolic extracts from the juice of *O. dillenii* showed weak to moderate antibacterial activities against the Gram-positive bacteria *Listeria monocytogenes* and the two Gram-negative bacteria species *Escherichia coli* and *Salmonella braenderup*. The MIC value of this extract against these bacterial strains was higher than 2.5 mg/mL.

6. Pancreatic α-Amylase and Intestinal α-Glucosidase Inhibitory Activities

The effects of the *O. dillenii* peel, seeds, and juice ethanolic extracts on the *in vitro* inhibition of pancreatic α-amylase and intestinal α-glucosidase activities are presented in Figure 5. Acarbose was used as a positive control. All the extracts had a significant inhibitory effect on the pancreatic α-amylase activity and this effect was dose-dependent. In general, the peel extracts of *O. dillenii* showed the greatest effect, followed by the juice and seed extracts. Concerning the *in vitro* intestinal α-glucosidase inhibition assay, results showed that the peel of *O. dillenii* also had the greatest inhibitory effect, followed by the juice and seeds.

7. Molecular Modeling Studies

To rationalize the antidiabetic activity results obtained and to investigate the putative mechanisms of action of the most abundant bioactive compounds present in *O. dillenii* peel ethanolic extracts, molecular docking studies against human pancreatic α-amylase enzyme were carried out. This method is considered as one of the most
popular and powerful theoretical tools utilized to provide an atomistic insight of the binding mode of a molecule into the active site of a given target protein/receptor [43, 44]. This knowledge plays a crucial role in the determination and understanding of the bioactivity efficiency of the molecule for use as a therapeutic agent [45]. The method is based on the calculation of the free-binding energy of the molecule-receptor complex. The complex with the lowest binding energy indicates the most putative active inhibitor.

### Table 7: Docking results against human pancreatic α-amylase enzyme.

| Compound   | Binding affinity (kcal/mol) | Involved residues                                                                 | Type of interactions                      |
|------------|----------------------------|----------------------------------------------------------------------------------|------------------------------------------|
| Gallic acid| −6.4                       | Asp300, Glu233, Asp197, Tyr62, His299, His101, Arg195, Trp58, Leu165, Ala198    | HB, Pi-anion, Pi-Pi stacked, VDW          |
| p-Coumaric acid| −6.4                      | Asp197, Trp59, Glu63, Arg195, Tyr62, Asp300, Trp58, His299, Leu165, Glu60     | HB, Pi-Pi stacked, UDD, VDW              |
| Kaempferol | −8.7                       | Asp300, His305, Trp59, Trp58, Glu233, Asp197, Tyr62, His299, His101, Arg195   | HB, Pi-anion, Pi-Pi stacked, VDW          |
| Catechin   | −8.8                       | Asp300, Asp197, Glu63, Tyr62, Trp59, Glu233, His299, His101, Arg195, Trp58, Leu165, Ala198, His305 | HB, Pi-anion, Pi-Pi stacked, VDW          |
| Naringin   | −10.0                      | Asp300, Glu233, His299, Arg195, Asp197, Tyr62, Trp59, His305, Lys200, Ala198, Ala307, Leu162, Trp58, Ser199, Val234, His201, Ile235, Tyr151 | HB, Pi-anion, Pi-Pi stacked, UAA, UDD, CHB, VDW |
| Chalcone   | −7.8                       | Gln63, Trp59, Tyr62, Asp197, His305, Leu165, Trp58                              | HB, Pi-Pi stacked VDW                     |
| Acarbose   | −8.2                       | Asp300, Asp353, Asp356, Glu233, Arg195, His101, His305, Tyr62, Asp197, His299, Ile235, Ala198, Leu162, Leu165, Trp58, Trp59, Trp357, Val354 | HB, UAA, CHB, VDW                       |

HB, Hydrogen bond; VDW, Van der Waals forces; CHB, carbon hydrogen bond; UAA, unfavorable acceptor-Acceptor; UDD, unfavorable donor-donor.

**Figure 7:** 2D-representations of the chemical bonding modes of the complexes formed between naringin, catechin, kaempferol, and acarbose compounds and the catalytic site residues of human pancreatic α-amylase.
7.1. Validation of Docking Protocol. The reliability of the docking protocol was checked by docking validation experiments. The native ligand (cocrystallized ligand) was redocked to the catalytic active site of the crystal structure of α-amylase. The validation results showed that cocrystallized and redocked ligands were almost superimposed and had similar conformational poses in the binding pocket of α-amylase protein with a RMSD value of 1.172 Å, which is lower than 2.0 Å (the maximum allowable RMSD value). These findings clearly indicate the validity of the docking method used in this work. The validation results are displayed in Figure 6.

7.2. Docking Analysis for the Inhibitory Mechanism against α-Amylase. The docking results of the selected phytochemicals toward α-amylase enzyme were assembled in Table 7. The results showed that naringin, catechin, and kaempferol compounds had high affinities towards the active pocket of α-amylase and even better than the reference standard drug acarbose used in this study. The binding energy values were estimated to be −10.0 kcal/mol for naringin, −8.8 kcal/mol for catechin and −8.7 kcal/mol for kaempferol, while acarbose showed a binding energy of just of −8.2 kcal/mol. Chalcone was found to exhibit a good binding score equal to −7.8 kcal/mol, while gallic acid and p-coumaric acid showed the same moderate score of −6.4 kcal/mol.

Further analysis of the interaction modes of the selected compounds was performed to explain the inhibition mechanism. These indicated that mainly hydrogen, hydrophobic, and electrostatic interactions were responsible for the binding of all molecules with the active site α-amylase amino acid residues. As depicted in Figure 7, naringin, which exhibited the lowest binding energy and hence the most putative active inhibitor, interacted with α-amylase by several interactions with surrounding residues. It constructed conventional hydrogen bonds with four amino acids, namely Asp300, Glu233, His299, and Arg195; one salt bridge with Asp197, one carbon hydrogen bond with Ala198 residue; two unfavorable donor-donor interactions with His299 and His305; and one unfavorable acceptor-acceptor contact with Lys200. This compound also formed two hydrophobic interactions as Pi-Pi stacked with Tyr62 and contact with Lys200. His305 and Asp300 were involved in the formation of hydrogen bonds. In addition, p-coumaric acid was found to be surrounded by several interactions with the receptor of all the compounds. These interactions included one hydrogen bond, two Pi-Pi stacked and four VDW interactions with the side chain amino acids Gln63, Trp59, Tyr62, Asp197, His305, Leu165, and Trp58, respectively. Gallic acid interacted with the residues Asp300, Glu233, Asp197, His299, His101, Arg195, Trp58, Leu165 and Ala198 via hydrogen bonds (two bonds), Pi-Anion (one interaction), Pi-Pi stacked (one interaction) and VDW (six interactions). In addition, p-coumaric acid was found to be surrounded by Asp197, Trp59, and Gln63 (via hydrogen bonds), Arg195 (unfavorable donor-donor interaction), Tyr62 (via Pi-Pi stacked interaction), and Asp300, Trp58, His299, Leu165, and Glu60 (via VDW forces) residues.

8. Discussion

In this research, extracts were prepared from different organs of O. dillenii fruits. Results showed high yield variability depending on the organ. This agreed with previous studies performed in different countries showing high variations in the ethanolic extract yields of Opuntia species. Indeed, yields between 3.93% and 6.25% were obtained with O. dillenii peel and juice from Taiwan [8]. This yield was between 6.20% and 15.5% for different Opuntia species from Mexico using the same extraction method. It was therefore suggested that extraction yields depend on genetic factors, harvesting routine, growing conditions, and cultivars and that the amounts of chemical compounds found in the extracts differed depending on the cultivar, climatology, harvesting conditions, and developing environment [46].

For each fruit organ, the mean quantity of total polyphenols and total flavonoids was determined. Results showed differences depending on both factors which could be explained by different characteristics, as it is known that they can be related to genotypic factors, biotic (physiological stage, organs and species) and abiotic (edaphic) [47, 48]. Results obtained in the present study agree with other works showing that the total polyphenol content of O. dillenii fruits is low [8, 49]. As an example, Betancourt et al. [50] found the highest total phenolic compound content in a crude O. dillenii fruit extract from Colombia (311.61 μg/100 mg), while Embaby et al. [51] showed that O. dillenii fruits from Egypt had a low phenolic content (179.30 μg/100 mg).

The different ethanolic extracts were then analyzed by HPLC for the determination of the major phenolic
molecules. The present research highlighted that seeds of *O. dillenii* fruits constitute a great source of flavonoids and polyphenols, which are important plant secondary metabolites [9]. For example, Katanic et al. reported low amounts of quercetin and kaempferol (0.6 and 2.7 mg/g, respectively) in the peel of this fruit [17]. In the work of Yilmaz and Toledo, the seeds presented the highest amount of polyphenols, followed by the peel and juice [7]. Another work reported the detection of quercetin, rutin, naringin, catechin, caffeic acid, gallic acid, chlorogenic acid, cinnamic acid, and syringic acid in *O. dillenii* fruits [52]. Also, Qiu et al. identified many phenolic compounds in the fruit, with 3-O-methyl quercetin, kaempferol and quercetin as the main phenols [53].

It is well known that most of these molecules display antimicrobial activities and that they can prevent chronic diseases such as cardiovascular disease, inflammation, and cancer [9]. Furthermore, some compounds such as kaempferol have antimicrobial and antioxidant properties [17, 54]. The variability of phenolic molecules in the extracts may influence their biological activities as the number and position of hydroxyl groups in the aromatic rings may influence their toxicity towards microorganisms. On the other hand, the antimicrobial activity is not only dependent on the presence of phenolic compounds but also on the presence of various other secondary metabolites.

Results obtained in the present study highlighted the interesting antibacterial activity of the extracts, which may be due to the presence of catechin and gallic acid, the major molecules in the active extracts. Indeed, it has been previously reported that these compounds possess antimicrobial activities. Gallic acid notably exhibits antibacterial capacity against various food-borne pathogens and food-borne harmful bacteria such as *Helicobacter pylori*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus mutans*, and *Listeria monocytogenes* [55–57]. However, the observed activities were lower than those of the positive standard, which may be due to the inability of the antimicrobial agent to diffuse uniformly into the agar, as has already been reported by many researchers [58, 59]. The low phenolic compound content in the studied extracts could also be responsible for the observed inefficiency of the peel and seeds extracts, as there is a positive correlation between the concentration of phenolic compounds and their antimicrobial activity [60], with polyphenols and flavonoids representing important antimicrobial substances. Therefore, we suggest that the ethanolic extracts obtained from the juice of *O. dillenii* could be used to purify compounds with antibacterial activities or as a food ingredient.

The *in vitro* anti-diabetic activities of the *O. dillenii* extracts were analyzed. One therapeutic approach for diabetes consists of reducing postprandial hyperglycemia by delaying glucose absorption by inhibiting carbohydrate hydrolyzing enzymes (α-amylase and α-glucosidase), thereby preventing an increase in postprandial glucose [61, 62]. Pancreatic α-amylase is a digestive enzyme that catalyzes the initial step of starch hydrolysis to maltose and ultimately glucose [63]. Alpha-glucosidases are digestive enzymes located at the brush edge of enterocytes that catalyze the hydrolysis of α-(1,4)-glycosidic bonds of food disaccharides that reach the jejunum. Residues from the digestion of starch lead to the formation of absorbable glucose by intestinal cells [64, 65]. As a result, much research has focused on the identification of potential α-amylase and α-glucosidase inhibitor compounds for the prevention and treatment of diabetes.

Results obtained here showed the interesting ability of all extracts to inhibit the activity of α-glucosidase and α-amylase, with peel extracts displaying the highest activities. The differences in the activities reported for the different extracts may be related to differences in their chemical compositions. According to the HPLC data, the main compounds in the peel extracts were quercetin, kaempferol, and naringin, each of which is known for its ability to inhibit the activity of α-glucosidase and α-amylase. Those molecules, and possibly other active compounds may inhibit enzymes that hydrolyze polysaccharides and disaccharides. They may be nonspecific, in which case they would inhibit both α-amylase and α-glucosidase. It has been suggested that there may be a correlation between the content in phenolic compounds and the ability to inhibit the activity of α-glucosidase and α-amylase [66–69]. However, plant extracts with a high polyphenol content do not always exert a high inhibitory activity on α-amylase [70] which highlights the importance of the nature of different molecules and the interactions between them [71]. In addition, there is also a reported correlation between flavonoids and the inhibition of α-glucosidase and α-amylase. Some of the molecular mechanisms by which these compounds exert anti-diabetic effects have already been evaluated [72–74]. These phenolic compounds, which are characterized by antioxidant activities, are known to have anti-diabetic activities by regulating the disturbed oxidative medium under diabetic conditions [75]. Tadera et al. evaluated the inhibitory activity of six groups of flavonoids on yeast and rat intestinal α-glucosidase and porcine pancreatic α-amylase. For pancreatic α-amylase inhibition, rutin, myricetin, and quercetin were the most potent compounds, showing inhibition percentages of 61%, 64%, and 50%, respectively. Quercetin, kaempferol, luteolin, naringin, and cyanidin showed inhibition percentages of 91%, 82%, 92%, 73%, and 99%, respectively, against the yeast α-glucosidase. Weak inhibitory activity was observed in the presence of intestinal α-glucosidase [61]. In addition, other authors have shown that catechins can inhibit the activity of pancreatic α-glucosidase and α-amylase [63, 76]. From those results, it can be concluded that the antihyperglycemic effect of *O. dillenii* is related to its richness in natural bioactive compounds that have an important antidiabetic property while reacting alone or in synergy.

On the other hand, molecular docking simulations of the predominating metabolites identified in *O. dillenii* peel ethanolic extract were performed toward the human pancreatic α-amylase enzyme. Results clearly indicated the presence of bioactive molecules with high binding affinities to the α-amylase. Interestingly, naringin, catechin, and kaempferol exhibited lowest binding energies and therefore highest activities than the standard drug acarbose. Furthermore, all the studied compounds were found to form
several hydrogen, hydrophobic, and electrostatic interactions with the same amino acid residues that interact with the cocrystallized ligand, which can explain the high stability of the complexes formed between these molecules and α-amylase. Investigation of the crystal structure of α-amylase demonstrated the presence of important residues in the active pocket, especially the three amino acids Asp300, Glu233, and Asp197 [77–79]. These three characteristic residues are the enzymes’ key residues that contribute to the overall catalytic activity of α-amylase enzyme [80, 81]. Brayer et al. [82] demonstrated by kinetic analyses that the substitution of Asp197 leads to a 106-fold decrease in catalytic activity of α-amylase enzyme, while the substitution of Glu233 and Asp300 residues decline the activity by 103-fold. As it can be noted from the docking results, most of the studied compounds showed interactions with these enzymes’ key residues and that may explain their strong binding at the active site, specifically naringin, catechin, and kaempferol compounds which have been already reported as potent antidiabetic agents [83–85]. Besides these observations, it is very exciting to note that the number of rings contained in the studied molecules increases the binding affinity to the receptor, hence the inhibitory potential. Overall, and based on the obtained observations, it can be concluded that the docking results are in accordance with the obtained in vitro experimental outcomes and demonstrate that O. dillenii ethanolic extract is rich in natural compounds that can be promising leads to developing highly active inhibitors against diabetes type 2, which would be better than the acarbose used currently. However, more theoretical and experimental validations are essential to determine and better understand the therapeutic effect of these compounds in the inhibition mechanism of diabetes.

9. Conclusions

The present study revealed that the extracts produced from seeds, juice, and peel of O. illeniid fruits have an interesting chemical composition, including the presence of high polyphenol quantities (193.73 ± 81.44 to 341.12 ± 78.90 gallic acid eq [g/100g dry weight]). Notably, gallic acid, vanillic acid, quercetin 3-O-β-D-glucoside, kaempferol, and syringic acid, which were the dominant constituents, are well known for their remarkable biological activities, including anti-diabetic, antibacterial, and antifungal properties [57, 60]. Results also showed that the yield, chemical composition, and biological activity of O. illeniid seeds, juice, and peel extracts varied according to the plant organ and extraction solvent, with the highest yields being obtained with ethanol extracts (8.41 ± 5.19%). In this study, a noteworthy anti-diabetic effect was highlighted for Opuntia extracts, in both in vitro and in silico assays, suggesting that they could potentially be used as a phytomedicine in healthy and diabetic subjects to prevent the incidence of diabetes and delay the development of complications in diabetics. The results of the in silico assays, which were consistent with the experimental outcomes, indicated the presence of naringin, catechin, and kaempferol as antidiabetic bioactive compounds. In addition, they provided a deeper insight into overall molecular interactions between the selected compounds and α-amylase enzyme, notably highlighting high-binding affinities. In conclusion, the study demonstrated that these natural metabolites in O. dillenii ethanolic extracts can be good alternatives to the standard approved drug acarbose.

As a perspective, it would be interesting to study the mechanisms of action of those extracts and their optimal dosages as well as their method of administration for the patients. In addition, these extracts could be used in the agrifood industry as additives, dyes, and natural food preservatives thanks to their antibacterial properties. Based on the chemical profile of O. dillenii fruits, bio-guided assays could be performed to isolate the main pharmacologically active constituents, which could then be used in the prevention and treatment of various diseases, representing one of the possible prospects for the food and nutraceutical industries. The high efficacy and decreased side effects of this plant highlight the possibility of using medicinal plants instead of chemical drugs. Moreover, this approach will provide great benefits for Moroccan farmers and increase their economic income.

Abbreviations

O. dillenii: Opuntia dillenii
LOD: Limit of detection
LOQ: Limit of quantification
MIC: Minimum inhibitory concentration
DNSA: 3,5-dinitrosalicylic acid
OD: Optic density
SD: Standard deviation
GAE: Gallic acid equivalent
DW: Dry weight
QE: Quercetin equivalent
HB: Hydrogen bond
VDW: Van der waals forces
CHB: Carbon hydrogen bond
UAA: Unfavorable acceptor-acceptor
UDD: Unfavorable donor-donor.

Data Availability

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

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Authors’ Contributions

Conceptualization was performed by E.H.L. and M.-L.F. Methodology was done by B.B., M.G., K.Z., and F.A. Data curation was done by M. A., M.B. (Mohammed Bouhrim) and F.A. Writing of the original draft was done by E.H.L. and B.B. Supervision was done by M.B. (Mohammed benouham), M.B. (Mohammed Bellaoui), B.H., and M.R. All authors have read and agreed to the published version of the manuscript.
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