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Phytochemical Characterization and Bioactivity of Extracts from Different Fruit Parts of *Opuntia leucotricha* DC.: A Comparison between a Conventional Organic Solvent and Green Natural Deep Eutectic Solvents

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Abstract: The objective of this study was to analyze the chemical profile of extracts from different fruit parts of the perennial arborescent cactus *Opuntia leucotricha* by applying ultra-high-performance liquid chromatography–high-resolution mass spectrometry (UHPLC-HRMS) and to evaluate their biological properties (antioxidant and enzyme inhibitory capacities). Extracts were obtained from the fruit pulp (without or with seeds), seeds, and peel by ultrasound-assisted extraction (UAE) using the conventional solvent methanol 50% and two Natural Deep Eutectic Solvents (NADESs) (glycerol:urea, 1:1 and citric acid:sorbitol, 1:2). A total of 33 compounds were identified, including phenolics, fatty acids, and others. Phenolic acids were the most abundant class of phenolics identified in all fruit parts, with the highest concentration observed in the methanol extracts from peel (593.02 $\mu g/g_{DW}$), followed by seed-containing pulp (69.03 $\mu g/g_{DW}$), pulp (57.83 $\mu g/g_{DW}$), and seeds (39.97 $\mu g/g_{DW}$). The second most effective extractant was NADES 1 (glycerol:urea, 1:1), which was also successful in extracting compounds with antioxidant capacity. Overall, the extracts demonstrated considerable enzyme inhibitory activities, with the greatest effects observed against α-amylase and α-glucosidase. The results indicate that *O. leucotricha* fruits could be a promising source of bioactive compounds, and NADES a viable alternative to organic solvents for their industrial exploitation.

Keywords: *Opuntia leucotricha* DC.; NADES; phenolic compounds; antioxidant capacity; enzyme inhibition; HPLC-HRMS

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1. Introduction

The genus *Opuntia* is a group of flowering succulent plants belonging to the Cactaceae family. They are distinguished by their ability to thrive and survive in dry environments,

particularly arid and semi-arid regions [1], due to their evolved adaptations to the Crassulacean Acid Metabolism (CAM) photosynthetic pathway and the production of spines and fleshy pads for water storage [2,3]. There are approximately 300 species of the genus *Opuntia*, with 104 species and varieties present in Mexico [4]. Among the numerous species of the *Opuntia* genus, *Opuntia leucotricha* DC. is distinguished as a perennial arborescent cactus of economic interest, native to arid and semi-arid regions of North America [5], particularly in the mountains of central Mexico [6]. This species has been introduced in a number of countries across the globe, including North Africa [7]. According to several authors, the *Opuntia* spp. were introduced to the Iberian Peninsula from the American continent, subsequently spreading throughout the Mediterranean Basin [8]. The initial introduction of *Opuntia* in Morocco was motivated by the desire to identify a crop that could withstand prolonged periods of drought and to protect houses and villages [9]. Subsequently, they have been extensively cultivated for their nutritional value and economic importance, given their numerous applications in agriculture and other industries, including the human diet, medicinal purposes, cosmetic uses, and as a source of natural pigments and forage [10].

The therapeutic and medicinal properties of bioactive compounds extracted from *Opuntia* spp. have attracted significant attention in recent years, as evidenced by the numerous studies conducted in this area [6,11,12]. Researchers have concentrated their efforts on the extraction of bioactive compounds from various parts of the *Opuntia* plant, including fruits, flowers, and cladodes [2]. The different parts of the fruit, namely the seeds, pulp, and peels, are considered a significant source of bioactive compounds with a wide range of biological activities, including antioxidant, anticancer, antimicrobial, antidiabetic, antihyperlipidemic, and neuroprotective, gastric, and diuretic effects [2,13]. The majority of the current industries continue to utilize conventional methods for the extraction of bioactive compounds from plants, which has a detrimental impact on both human health and the environment [14]. Consequently, there is a growing interest in the field of green chemistry in the use of Natural Deep Eutectic Solvents (NADES), which are composed of non-toxic materials and offer an alternative to traditional extraction methods [15]. The utilization of NADES for extraction not only provides a more sustainable approach but also offers advantages, including an enhanced extractability of compounds (e.g., phenolics) in comparison to conventional methods [16]. Furthermore, NADES are biocompatible, easy to prepare, have insignificant volatility and high dissolvability, and present high biodegradability and reduced toxicity [17].

Despite the abundance of *O. leucotricha* compared to other species of the *Opuntia* genus, there are no studies in the literature related to its bioactive compounds or biological activities. In this study, a green extraction method was employed to extract the compounds from different fruit parts of *O. leucotricha*, namely the peel (Pe), seeds (S), pulp without seeds (P), and seed-containing pulp (SP). For this purpose, ultrasound-assisted extraction (UAE) was employed, utilizing two distinct NADES (glycerol:urea, 1:1 and citric acid:sorbitol, 1:2). A conventional solvent (methanol 50%) was employed as a control. To the best of our knowledge, this is the first study to investigate the antioxidant properties of *O. leucotricha* and its capacity to inhibit the enzymes linked with human pathologies, such as acetylcholinesterase, butyrylcholinesterase, tyrosinase, α-glucosidase and α-amylase. Furthermore, this study represents the inaugural investigation utilizing NADES for the extraction of biocompounds from the *Opuntia* genus.

2. Materials and Methods

2.1. Chemicals and Reagents

Glycerol, quercetin ($C_{15}H_{10}O_7$), vanillin ($C_8H_8O_3$), catechin ($C_{15}H_{14}O_6$), rutin (quercetin 3-rutinoside) ($C_{27}H_{30}O_{16}$), hyperoside (quercetin-3-galactoside) ($C_{21}H_{20}O_{12}$), isorhamnetin 3-glucoside ($C_{22}H_{22}O_{12}$), narcissin (isorhamnetin 3-rutinoside) ($C_{28}H_{32}O_{16}$), nicotiflorin (kaempferol-3-O-rutinoside) ($C_{27}H_{30}O_{15}$), eriodictyol (5,7,3′,4′-tetrahydroxyflavanone) ($C_{15}H_{12}O_6$), isoquercitrin (quercetin 3-glucoside) ($C_{21}H_{20}O_{12}$), protocatechuic acid (3,4-dihydroxybenzoic acid) ($C_7H_6O_4$), vanillin (4-hydroxy-3-methoxybenzaldehyde) ($C_8H_8O_3$),

4-hydroxybenzoic acid ($C_7H_6O_3$), syringic acid (4-hydroxy-3,5-dimethoxybenzoic acid) ($C_9H_{10}O_5$), 2,2-diphenyl-1-picrylhydrazyl (DPPH) ($C_{18}H_{12}N_5O_6$), trichloroacetic acid (TCA), potassium persulfate ($K_2S_2O_8$), 2,2′ -azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt tablets (ABTS), dibasic sodium phosphate anhydrous (Na_2HPO_4), tyrosinase, kojic acid ($C_6H_60_4$), 3,4-dihydroxy-L-phenylalanine (L-DOPA), 5,5-dithiobis(2-nitrobenzoic acid) (DTNB) ($C_{14}H_8N_2O_8S_2$), acetylthiocholine iodide (ATCI) ($C_7H_{16}NOS^+$), butyrylthiocholine chloride (BTCI) ($C_9H_{20}NOS^+$), electric-eel acetylcholinesterase (AChE), horse-serum butyrylcholinesterase (BChE), galanthamin ($C_{17}H_{21}NO_3 \cdot HBr$), *p*-nitrophenyl-α-D-glucopyranoside, α-glucosidase, α-amylase from porcine pancreas, and 3,5-dinitrosalic ylic acid (DNS) were obtained from Sigma–Aldrich (Steinheim, Germany). *p*-Coumaric acid ($C_9H_8O_3$), sinapic acid (3,5-dimethoxy-4-hydroxycinnamic acid) ($C_{11}H_{12}O_5$), and taxifolin (2,3-dihydroquercetin) ($C_{15}H_{12}O_7$) were obtained from AASC Ltd. (Southampton, UK). Dihydroferulic acid (3-(4-hydroxy-3-methoxyphenyl) propanoic acid) was purchased from Alfa Aesar (Lancashire, UK). Citric acid ($C_6H_8O_7$), sorbitol ($C_6H_{14}O_6$), ethanol (C_2H_5OH), aluminum chloride ($AlCl_3$), and naringenin (5,7,4′-trihydroxyflavanone) ($C_{15}H_{12}O_5$) were purchased from Fluka (Buchs, Switzerland). Dibasic potassium phosphate (K_2HPO_4), sodium acetate (CH_3COONa), fluorescein ($C_{20}H_{12}O_5$), monobasic sodium phosphate (NaH_2PO_4), and starch from potato ($C_6H_{10}O_5$) were provided by Panreac (Barcelona, Spain). Potassium hexacyanoferrate (III) ($K_3[Fe\ (CN)_6]$), (±)-6-Hydroxy-2,5,7,8-tetramethylchroma ne-2-carboxylic acid (Trolox) ($C_{14}H_{18}O_4$), 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH), and choline chloride were obtained from Acros Organics (Geel, Germany). Folin–Ciocalteu reagent, sodium carbonate (Na_2CO_3), gallic acid ($C_7H_6O_5$), and iron (III) chloride ($FeCl_3$) were acquired from VWR (Leuven, Belgium). Urea (NH_2CONH_2) and methanol (CH_3OH) were purchased from Fisher Scientific (Leicestershire, UK). Monobasic potassium phosphate (KH_2PO_4) and ascorbic acid ($C_6H_80_6$) were obtained from Merck (Darmstadt, Germany).

2.2. Plant Material

The plants of *Opuntia leucotricha* DC. sampled in this study grow spontaneously in the mountains of northern Morocco. The fruits were harvested in August 2021 from the rural commune of Saddina, Province of Tetouan (Tangier-Tetouan-Al Hoceïma region). The fruit is characterized by a green peel, an orange edible portion, and a sweet taste. The fruits were separated into four distinct categories: peel (Pe), seeds (S), pulp without seeds (P), and seed-containing pulp (SP). The plant material was dried in a ventilated oven at 40 °C until the weight of the dry matter had stabilized. It was then crushed using an electric grinder. The crushed material was then sieved through a stainless-steel sieve with 0.2 mm diameter (Fisher Scientific LABOSI, Elancourt, France) to obtain a homogeneous powder. Subsequently, the powder was then stored in hermetically sealed flasks at room temperature in the dark until analysis.

2.3. NADES Preparation

The preparation of NADES was based on the heating and stirring method, as previously described in reference [14]. Two distinct NADES mixtures were employed in the present study: glycerol:urea, 1:1 (Gly:U, 1:1, NADES 1) and citric acid:sorbitol (CA:S, 1:2, NADES 2). The mixture of two components with 30% water was placed in a flask and heated at a constant temperature (50–80 °C) using a magnetic stirrer for 30–90 min until a homogeneous, clear liquid was formed [18]. The quantity of water was employed to reduce the viscosity of the mixture, which is a significant challenge in the production of NADES, in order to enhance the extraction yield of bioactive compounds [19].

2.4. Ultrasound-Assisted Extraction (UAE)

We added 250 mg of powder from each fruit part to 10 mL of the extraction solvent (methanol 50%, NADES 1, and NADES 2), using separate Erlenmeyer flasks with a volume capacity of 100 mL. Subsequently, the mixture was subjected to ultrasonic waves for a

period of 60 min at 50 °C. The extracts were then filtered through Whatman n°. 1 filter paper (Whatman Int. Ltd., Maidstone, UK) and stored at −20 °C until use.

2.5. Phytochemical Analysis of the Extracts

2.5.1. Total Phenolic Content (TPC) Determined by the Folin–Ciocalteu (F-C) Method

The total phenolic contents were determined according to Ainsworth et al. [20], using a spectrophotometric method with Folin–Ciocalteu (F-C) reagent. Briefly, 200 µL of F-C reagent 10% (*v*/*v*) was mixed with 100 µL of each extract diluted in 75 mM phosphate buffer (pH 7.0) and 800 µL of 700 mM Na_2CO_3. The mixture was incubated for 2 h at room temperature in the dark. The absorbance of the reaction mixture was measured at 765 nm. A calibration curve was built with different concentrations of gallic acid, in order to calculate the total phenolic contents, and the results were expressed as milligrams of gallic acid equivalents per gram of dry weight (mg_{GAE}/g_{DW}).

2.5.2. Total Flavonoid Content (TFC) Determined by the Aluminum Chloride Method

The total flavonoid content was determined using the aluminum chloride method of Chang et al. [21]. Briefly, 250 µL of sample dilution was mixed with 750 µL of 80% ethanol, 50 µL of 1% $AlCl_3$ in ethanol 80%, and 50 µL 1 M sodium acetate. The mixture was incubated for 30 min at room temperature. The absorbance of the reaction mixture was measured at 415 nm. A calibration curve was prepared using quercetin as a standard, and the results were expressed as milligrams of quercetin equivalents per gram of dry weight (mg_{QE}/g_{DW}).

2.5.3. Total Tannin Content (TTC) Determined by the Vanillin/HCl Method

The tannin contents were determined using the vanillin/HCl method [22]. Just before the reaction, the reagent was prepared by mixing equal portions of 8% HCl and 1% vanillin, both in methanol. Then, 100 µL of extracts was mixed with 200 µL of vanillin/HCl reagent in a microplate well. After incubation at 30 °C for 20 min, the absorbance was recorded at 500 nm. A calibration curve was obtained using catechin, and the results were expressed as milligrams of catechin equivalents per gram of dry weight (mg_{CE}/g_{DW}).

2.5.4. UHPLC-HRMS Analysis

An Ultimate 3000 RS UHPLC system (Dionex, San José, CA, USA) was used to conduct the chromatographic analysis of the compounds extracted from the peel, seed, pulp, and seed-containing pulp of the fruits of *O. leucotricha* using methanol 50%, NADES 1 (Gly:U, 1:1), and NADES 2 (CA:S, 1:2), as described previously in Cáceres-Jiménez et al. [23]. Each extract was filtered through 0.45 µm pore cellulose acetate membranes (VWR international, Radnor, PA, USA). A Zorbax SB-C18 RRHD column of 100 × 2.1 mm i.d., 1.8 µm (Agilent, Santa Clara, CA, USA) was preceded by a guard precolumn of the identical stationary phase. The entire setup was maintained at 40 °C for the separation of phenolic compounds using HPLC. The flow rate of 0.2 mL/min was established alongside a 26 min gradient of phase A: deionized water containing 0.1% formic acid and B: acetonitrile containing 0.1% formic acid. The gradient commenced at 3% B, was maintained for 2 min, then increased to 65% B in 18 min, before rising to 80% B in 1 min and being constant for 6 min, with a 26 min gradient. Afterward, the column was brought back to the initial conditions within 10 min. The Exactive Orbitrap mass spectrometer, equipped with a heated electrospray ionization probe (ThermoFisher Scientific, San José, CA, USA), functioned in the negative ionization mode (scanning from 100 to 1000 m/z). The capillary temperature was set at 300 °C, while the heater temperature was maintained at 150 °C. The sheath gas and auxiliary gas flowed at 20 units each, the sweep gas at 3 units, and the spray voltage was maintained at 4.00 kV. Data acquisition and processing were conducted using Xcalibur (3.0 software).

Phenolic compounds were identified in two ways: when standards were commercially available, the exact mass and the retention time (RT) were compared with them, and if the standards were unavailable, the identification of polyphenols was tentatively attained by

comparing the theoretical exact mass of the molecular ion with its accurately measured mass. Afterward, they were assigned to various libraries and open-access databases containing precise mass spectral information (HRMS), among others the Phytohub, phenol Explorer, and Metlin databases. Moreover, the identification of compounds was carried out following the MSIMS levels previously established by [24]. The chemical formulas, theoretical accurate mass, retention time (Rt), and delta error (ppm) between the theoretical accurate mass and the mass found of the detected compounds, as well as MSI MI levels, are presented in Supplementary Table S1. Standard calibration curves (different ranges between 0.01 and 140 mg/L) were used for the quantification of compounds. In case of the absence of reference compounds, the quantification was performed using a closely related parent compound. The different parameters used in the quantification of the chemical compounds are listed in Supplementary Table S2. All the analyses were performed in duplicate.

2.6. Antioxidant Capacity

2.6.1. ABTS Free Radical Scavenging Assay

The ability of the extracts to neutralize ABTS free radicals was evaluated by applying the assay described by Re et al. [25]. To prepare the ABTS radical cation, a 10 mg ABTS tablet was mixed with 2.6 mL $K_2S_2O_8$ in distilled water to give a final concentration of 7 mM, and the stock solution was kept in the dark at room temperature for 12–16 h before use. In order to obtain the working solution, the basic solution was diluted with water until the absorption value of 0.7 ± 0.02 was reached at 734 nm. The reaction was begun by adding 10 µL of each extract to 190 µL of the test reagent in a clear 96-well microplate, and the absorbance was measured immediately at 734 nm. Trolox was used as the standard, and the results were expressed as milligrams of Trolox equivalents per gram of dry weight (mg_{TE}/g_{DW}).

2.6.2. DPPH Free Radical Scavenging Assay

The DPPH assay was performed as described by Soler-Rivas et al. [26] with slight modifications. The assay is based on mixing 10 µL of the plant extract, 100 µL of 90 µM DPPH, and 190 µL of methanol 80%. The mixture was read at 515 nm after 30 min incubation at room temperature in the dark using a microplate reader. Trolox was used as the standard, and the results were expressed as milligrams of Trolox equivalents per gram of dry weight (mg_{TE}/g_{DW}).

2.6.3. Ferric Reducing Antioxidant Power (FRAP)

For the FRAP assay, the procedure described by Yen et al. [27] was used. This method is based on the ability of antioxidant compounds of the extract to reduce ferric to ferrous ions. We incubated 100 µL of the extract, 250 µL of $K_3[Fe(CN)_6]$ solution (1%), and 250 µL of potassium phosphate buffer (200 mM, pH 6.6) for 20 min at 50 °C. The mixture was centrifuged for 10 min after the addition of 250 µL of 10% TCA. Then, 100 µL of the supernatant, 100 µL of the water, and 20 µL of 0.1% $FeCl_3$ were mixed, and the absorbance was read at 700 nm to determine the amount of ferric ferrocyanide formed. Ascorbic acid was used as standard, and the results were expressed as milligrams of ascorbic acid equivalents per gram of dry weight (mg_{AAE}/g_{DW}).

2.6.4. Oxygen Radical Absorbance Capacity (ORAC) Assay

The oxygen radical absorbance capacity (ORAC) assay measures the ability of an antioxidant to quench free radicals by hydrogen donation; it is a measure of both general and specific antioxidant actions, and it was performed based on the method described by Gillespie et al. [28]. A black microplate containing 150 µL of 0.08 µM fluorescein solution and 25 µL of the plant extracts was preheated for 10 min at 37 °C. Then, 25 µL of AAPH solution was added to each well and the kinetic read began immediately. The microplate reader was set to run a fluorescence kinetic read with an excitation wavelength of 485 nm

and an emission wavelength of 530 nm. The assay's temperature was at 37 °C for 90 min and the read was every 5 min up to the value zero of the fluorescence. The results were calculated using the differences in areas under the fluorescein decay curve (AUC) between the blank (without plant extract) and the sample. The final ORAC values were calculated using the regression equation between Trolox equivalents and the net AUC. Trolox (6.25–50 µM) was used as the standard, and the results were expressed as milligrams of Trolox equivalents per gram of dry weight (mg_{TE}/g_{DW}).

2.7. Enzyme Inhibitory Activities

2.7.1. Acetylcholinesterase (AChE) and Butyrylcholinesterase (BChE) Inhibitions

The inhibition of AChE and BChE was performed according to Ellman et al. [29]. Briefly, 25 µL of diluted extract (1:2) was mixed with 125 µL of 3 mM DTNB, 50 µL of 100 mM phosphate buffer (pH 8.0), and 25 µL of 15 mM substrate (ATCI or BTCI). Then, 25 µL of enzyme (AChE or BChE) was added to the mixture, and the absorbance was read immediately at 405 nm and after 5 min. Galanthamine (25 µg/mL) was used as a positive control, and the results were expressed in % inhibition.

2.7.2. Tyrosinase (Tyr) Inhibition

Based on Masuda et al. [30], the inhibition of Tyr was carried out as follows: a mixture of 40 µL of diluted extract (1:5), 40 µL of tyrosinase solution, and 80 µL of phosphate buffer was incubated for a period of 10 min; then, 40 µL of L-DOPA was added before a second incubation period of 10 min at room temperature; and the absorbance was measured at 475 nm. Kojic acid (200 µg/mL, in buffer) was used as the positive control, and the results were expressed in % inhibition.

2.7.3. Inhibition of α-Glucosidase and α-Amylase

The α-glucosidase and α-amylase inhibitory activities of the methanolic extracts were determined according to the Kwon et al. [31] method. For α-glucosidase, 50 µL of diluted extract (1:2) was incubated with 100 µL of enzyme solution (1.0 U/mL) at room temperature for 10 min. The absorbance was read at 405 nm before adding 50 µL of 5 mM *p*-nitrophenyl-α-D-glucopyranoside solution and again after incubating the reaction mixture at room temperature for 5 min. For the inhibition of α-amylase, a mixture of 40 µL of diluted extract (1:2), 160 µL of buffer, and 200 µL of enzyme solution was incubated for 5 min at room temperature before adding 400 µL of starch solution, and 3 min after, it was incubated for 15 min in a water bath at 85 °C after adding 400 µL of DNS reagent. Then, the absorbance was read at 540 nm and the α-amylase inhibitory activity was calculated.

2.8. Statistical Analysis

All tests were conducted in triplicate and data represent the mean ± standard error for the total number of experimental results. Data were analyzed by one-way analysis of variance (ANOVA), and Duncan's new multiple range test ($p < 0.05$) using IBM SPSS Statistics for Windows, Version 29.0.0.0 (241). Correlations were calculated using Pearson's test, and principal component analysis (PCA) was performed employing Origin pro graphing analysis ($p \leq 0.05$).

3. Results and Discussion

3.1. Effect of Solvent on Total Phenolic, Flavonoid, and Tannin Contents

The total phenolic contents (TPCs) of the extracts from different fruit parts, obtained using methanol 50% and two different deep eutectic solvents (NADES), are displayed in Figure 1a. The results demonstrate significant variations ($p < 0.05$) across different parts of the fruit and different extraction solvents. Irrespective of the solvent employed, and when fruit parts are compared, the TPCs were found to be considerably higher in the peel than in the other parts. This finding is consistent with other studies, such as El Mannoubi et al. [32] report, in which examined the peel and pulp of Tunisian red and

yellow–orange *Opuntia ficus indica* fruits using three different solvents (methanol 80%, ethanol 80%, and acetone 80%). Regardless of the solvent used, the peel consistently exhibited a higher TPC. Yeddes et al. [33] also reported similar results when analyzing the fruits of two *Opuntia* species from Tunisia, two forms of *O. ficus indica*, namely spiny (green–yellow peel and yellow pulp) and thornless (green peel and red-purple pulp), and *Opuntia stricta* (purple peel and pulp). A similar result was observed on the pulp and peel of *O. ficus indica* fruits from Egypt [34]. Conversely, seeds were found to be the fruit component with the lowest TPCs. In comparison to methanol 50%, both NADES demonstrated superior TPC results for this fruit part. NADES 2 exhibited the most favorable outcomes, with an average of 6.23 ± 0.06 mg_{GAE}/ g_{DW}. Conversely, the highest TPCs were observed for methanol 50% in the pulp, seed-containing pulp, and peel extracts. Pulp was the fruit part in which the solvent showed more of an influence on phenolic extraction.

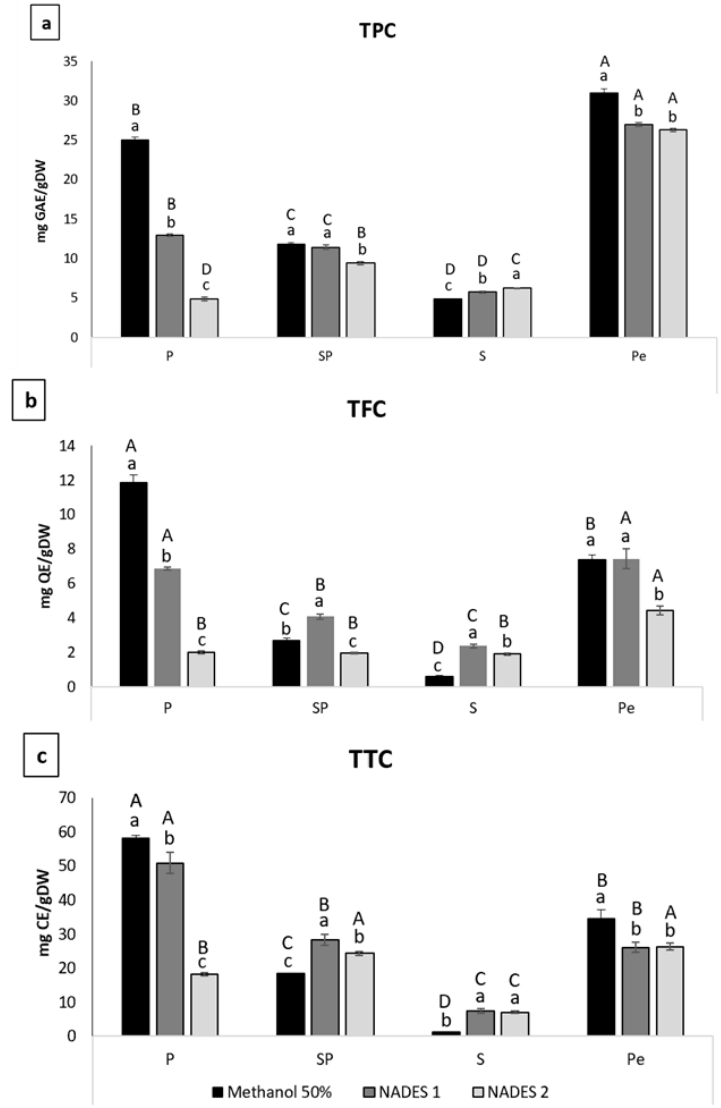

Figure 1. Total phenolic (TPC) (**a**), flavonoid (TFC) (**b**), and tannin (TTC) (**c**) contents of extracts from different fruit parts (P, pulp; SP, seed-containing pulp; S, seeds; and Pe, peel Pe) of *O. leucotricha* obtained with different extraction solvents (methanol 50%; NADES 1, glycerol:urea, 1:1; NADES 2, citric acid:sorbitol, 1:2). Values are expressed as mean ± SE (n = 3). Different letters in each series indicate significant differences ($p < 0.05$) (Duncan's new multiple range test). Uppercase letters indicate significant differences ($p < 0.05$) between the four parts of the fruits for each solvent studied, while a lowercase letter denotes significant differences ($p < 0.05$) between solvents for each independent fruit part.

In this study, the highest TFC was observed in methanol extract from pulp (11.85 ± 0.47 mg_{QE}/ g_{DW}). Except from this fruit part, NADES 1 was the best solvent to extract flavonoids from the other parts of *O. leucotricha* fruits (seed-containing pulp, seeds, and peel) (Figure 1b). Similarly, the highest TTC was observed in the pulp when using methanol 50% as the solvent (58.03 ± 0.91 mg_{CE}/ g_{DW}), followed by NADES 1 (50.87 ± 3.03 mg_{CE}/ g_{DW}). These findings differ from those reported by Ndhlala et al. [35], who demonstrated that the peel of *Opuntia megacantha* contains a higher concentration of tannins than the pulp. Furthermore, methanol 50% was identified as the optimal solvent for tannin extraction from the peel. However, both NADES 1 and NADES 2 demonstrated a superior performance in extracting these compounds from seed-containing pulp and seeds (Figure 1c).

3.2. Chemical Analysis of Extracts by UHPLC-HRMS

In the present study, UHPLC-HRMS was employed to characterize the chemical compounds in the methanol 50%, NADES 1 (Gly:U, 1:1), and NADES 2 (CA:S, 1:2) extracts derived from the pulp, seed-containing pulp, seeds, and peels of *O. leucotricha*. The chemical profile revealed the presence of 10 phenolic acids (cinnamic acids and benzoic acids), 12 flavonoids (flavanonols, flavanones, and flavonols), 4 lignans, and 2 other phenolic compounds in the extracts (Table 1). Furthermore, four fatty acids and a compound derived from glucose oxidation (gluconic acid) were also identified and quantified.

Phenolic acids belonging to the benzoic acid family constituted the most abundant phenolic compounds in the extracts derived from the four fruit parts. Piscidic acid, a phenolic compound present in high concentrations in pulp, seed-containing pulp, and particularly in peel extracts (120.045 ± 5.115 to 254.66 ± 28.81 $\mu g/g_{DW}$), was extracted in the highest quantity using methanol 50%. These findings are consistent with those of other studies on *Opuntia* spp., such as [36], in which this compound was found in higher quantities in the pulp and peel (approximately ten times more than in pulp) of four varieties of *O. ficus indica* from the Canary Islands, using methanol 50% as solvent. This compound has been identified as a key contributor to the antioxidant stress response observed in this fruit [37]. Dihydroferulic acid glucuronide isomer II was identified as a phenolic compound present in significant quantities in the pulp, seed-containing pulp, and peel. Sinapic acid was the most abundant cinnamic acid, with a particularly high concentration in the peel extract. Overall, the peel exhibited the greatest concentration of phenolic acids when compared with the other fruit parts. These findings are consistent with previous studies conducted by other authors on fruits of *Opuntia* spp., particularly *O. ficus indica* [36,38]. Phenolic acids were the most prevalent class of phenolic compounds identified, in accordance with the findings of other authors [39,40] on the fruits of *O. ficus indica*, while this was not the case for the fruits of *Opuntia engelmannii* collected in Bragança, Portugal [41].

Flavonoids are predominantly found in the peel, with naringenin, a flavanone, being the most abundant. These findings align with those of Belhadj Slimen et al. [42] on *O. ficus indica*. The highest extraction of this compound was obtained with NADES 2 (CA:S, 1:2), at 17.75 ± 0.70 $\mu g/g_{DW}$. The other two most abundant flavonoids found in the peel were the flavonols narcissin (isorhamnetin 3-O-rutinoside) (8.97 ± 1.37 $\mu g/g_{DW}$) and isorhamnetin rutinoside-rhamnoside (5.895 ± 0.745 $\mu g/g_{DW}$). The extraction of lignans was more effective when methanol 50% was used. In particular, syringaresinol hexoside isomer II was the most abundant, especially in the peel and seed-containing pulp extracts. Fatty acids exert a profound influence on the chemical profile of *O. leucotricha* fruit, particularly the trihydroxy octadecenoic acid (pinellic acid) and the octadecanedioic acid present in seed extracts.

Table 1. Qualitative and quantitative ($\mu g/g_{DW}$) analysis by High-Performance Liquid Chromatography–High-Resolution Mass Spectrometry (HPLC-HRMS) of the chemical profile of extracts from pulp, seed-containing pulp, seeds, and peel of *O. leucotricha* fruits obtained with methanol and NADES extracts.

Fruit Part	**Pulp**			**Seed-Containing Pulp**			**Seeds**			**Peel**		
Solvent	**MeOH 50%**	**NADES 1**	**NADES 2**	**MeOH 50%**	**NADES 1**	**NADES 2**	**MeOH 50%**	**NADES 1**	**NADES 2**	**MeOH 50%**	**NADES 1**	**NADES 2**
Phenolic acids												
Cinnamic acids												
Coumaric acid hexoside	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.07 ± 0.05^{a}	0.46 ± 0.01^{b}	0.47 ± 0.02^{b}
Sinapic acid hexoside	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.06 ± 0	n.d.	n.d.
Sinapic acid	0.85 ± 0.11^{C}	n.d.	n.d.	1.23 ± 0.01^{B}	n.d.	n.d.	0.25 ± 0.02^{D}	n.d.	n.d.	7.42 ± 0.14^{aA}	4.60 ± 0.03^{c}	5.65 ± 0.09^{b}
Total cinnamic acids	0.85^{B}	n.d.	n.d.	1.23^{B}	n.d.	n.d.	0.25^{C}	n.d.	n.d.	8.55^{aA}	5.07^{c}	6.12^{b}
Benzoic acids												
Protocatechuic acid	<LOQ	n.d.	n.d.	0.06 ± 0.01^{B}	n.d.	<LOQ	0.98 ± 0.03^{aA}	0.10 ± 0.00^{c}	0.22 ± 0.02^{b}	$0.055 \pm 0.005_{B}$	n.d.	0.05 ± 0.03
Piscidic acid	29.2 ± 0.31^{aB}	1.22 ± 0.32^{cB}	7.01 ± 0.49^{bB}	32.0 ± 0.19^{aB}	10.6 ± 0.14^{bB}	6.01 ± 0.50^{cB}	9.97 ± 0.14^{aB}	3.36 ± 0.22^{bB}	3.39 ± 0.20^{bB}	254 ± 28^{aA}	120 ± 5^{bA}	123 ± 8^{bA}
4-Hydroxybenzoic acid	0.06 ± 0.00^{B}	n.d.	0.19 ± 0.01	0.15 ± 0.02^{B}	0.11 ± 0.01^{B}	n.d.	1.22 ± 0.08^{aA}	0.48 ± 0.06^{bA}	0.23 ± 0.01^{b}	0.12 ± 0.00^{aB}	0.07 ± 0.02^{bB}	0.06 ± 0.00^{b}
Eucomic acid	9.77 ± 0.33^{aB}	0.48 ± 0.06^{cB}	3.97 ± 0.08^{bB}	11.4 ± 0.22^{aB}	4.55 ± 0.04^{bB}	3.49 ± 0.36^{bB}	2.34 ± 0.06^{aB}	0.26 ± 0.03^{bB}	0.38 ± 0.10^{bC}	97 ± 9.4^{aA}	53.2 ± 4.21^{bA}	58.8 ± 0.95^{bA}
Dihydroferulic acid glucuronide isomer I	2.48 ± 0.17^{B}	n.d.	n.d.	2.51 ± 0.29^{B}	n.d.	n.d.	3.62 ± 0.26^{B}	n.d.	0.56 ± 0.05	$24.04 \pm 0.83_{aA}$	9.46 ± 0.52^{b}	9.6 ± 0.52^{b}
Dihydroferulic acid glucuronide isomer II	15.5 ± 1.21^{aC}	1.10 ± 0.22^{bB}	2.23 ± 0.19^{bB}	$21.35 \pm 1.90_{aB}$	4.92 ± 0.21^{bB}	2.28 ± 0.47^{bB}	21.1 ± 0.45^{aB}	3.39 ± 0.56^{bB}	3.43 ± 0.74^{bB}	207 ± 1.22^{aA}	102 ± 10^{bA}	97 ± 8^{bA}
Hydroxybenzoic acid derivative	0.15 ± 0.01^{C}	n.d.	0.3 ± 0.08^{B}	0.40 ± 0.02^{aB}	0.57 ± 0.18^{aB}	0.23 ± 0.01^{aB}	0.42 ± 0.01^{aB}	0.64 ± 0.03^{aB}	0.63 ± 0.11^{aB}	0.63 ± 0.07^{bA}	1.78 ± 0.19^{aA}	$1.18 \pm 0.19_{abA}$
Total benzoic acids	56.98^{aB}	2.79^{cD}	13.69^{bB}	67.8^{aB}	20.73^{bB}	12.02^{cBC}	39.72^{aB}	8.24^{bC}	8.88^{bC}	584.465^{aA}	287.37^{bA}	291.245^{bA}
Total Phenolic Acids	57.83^{aB}	2.79^{cD}	13.69^{bB}	69.03^{aB}	20.73^{bB}	12.02^{cBC}	39.97^{aB}	8.24^{bC}	8.88^{bC}	593.02^{aA}	292.44^{bA}	297.37^{bA}
Flavonoids												
Flavanonols												
Taxifolin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.075 ± 0.005	n.d.	0.075 ± 0.005
Flavanones												
Eriodictyol	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.10 ± 0.00	n.d.	0.25 ± 0.02
Naringenin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	5.08 ± 0.27	n.d.	17.7 ± 0.70
Total flavanones	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	5.19	n.d.	18.005
Flavonols												
Isorhamnetin rutinoside-hexoside	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.36 ± 0.05^{a}	0.05 ± 0.00^{b}	0.08 ± 0.00^{b}
Kaempferol rhamnosyl-rhamnosyl-hexoside	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.005 ± 0.001	n.d.	n.d.	0.21 ± 0.04	n.d.	n.d.
Isorhamnetin rutinoside-rhamnoside	n.d.	n.d.	n.d.	0.02 ± 0.00^{B}	n.d.	n.d.	0.07 ± 0.00^{B}	n.d.	n.d.	5.89 ± 0.74^{aA}	0.56 ± 0.04^{c}	2.54 ± 0.01^{b}
Rutin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.06 ± 0.00	n.d.	n.d.	2.44 ± 0.37	n.d.	1.31 ± 0.09
Hyperoside	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.21 ± 0.01	n.d.	0.11 ± 0.02
Isoquercitrin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.03 ± 0.00	n.d.	n.d.	0.46 ± 0.02	n.d.	0.12 ± 0.02
Nicotiflorin	n.d.	n.d.	n.d.	0.004 ± 0.000^{B}	n.d.	n.d.	0.02 ± 0.00^{B}	n.d.	n.d.	1.17 ± 0.18^{aA}	0.15 ± 0.02^{b}	0.55 ± 0.07^{b}
Narcissin	0.03 ± 0.01^{B}	n.d.	n.d.	0.03 ± 0.00^{B}	n.d.	n.d.	0.09 ± 0.02^{B}	n.d.	n.d.	8.97 ± 1.37^{aA}	1.16 ± 0.13^{c}	4.89 ± 0.2^{b}
Isorhamnetin-3-O-glucoside	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.11 ± 0.01	n.d.	0.05 ± 0.01
Total flavonols	0.03^{B}	n.d.	n.d.	0.05^{B}	n.d.	n.d.	0.29^{B}	n.d.	n.d.	19.845^{aA}	1.93^{c}	9.67^{b}

Table 1. *Cont.*

Fruit Part	Pulp			Seed-Containing Pulp			Seeds			Peel		
Solvent	**MeOH 50%**	**NADES 1**	**NADES 2**	**MeOH 50%**	**NADES 1**	**NADES 2**	**MeOH 50%**	**NADES 1**	**NADES 2**	**MeOH 50%**	**NADES 1**	**NADES 2**
Total Flavonoids	0.03 ^{B}	n.d.	n.d.	0.05 ^{B}	n.d.	n.d.	0.29 ^{B}	n.d.	n.d.	25.11 ^{aA}	1.93 ^{b}	27.75 ^{a}
Lignans												
Syringaresinol hexoside isomer I	0.47 ± 0.05 ^{B}	n.d.	n.d.	3.61±0.07 ^{A}	n.d.	n.d.	2.93 ± 0.25 ^{AB}	n.d.	n.d.	5.44 ± 1.38 ^{A}	1.51 ± 0.10	n.d.
Secoisolariciresinol hexoside isomer I	0.08±0.00 ^{C}	n.d.	n.d.	0.59 ± 0.03 ^{aA}	0.07 ± 0.01 ^{b}	0.01 ± 0.01 ^{b}	0.31 ± 0.01 ^{B}	0.01 ± 0.01	n.d.	0.07 ± 0.00 ^{C}	n.d.	n.d.
Secoisolariciresinol hexoside isomer II	0.16 ± 0.02 ^{B}	n.d.	n.d.	0.21 ± 0.02 ^{A}	n.d.	n.d.	0.12±0.00 ^{B}	n.d.	n.d.	0.05 ± 0.00 ^{C}	n.d.	n.d.
Syringaresinol hexoside Isomer II	2.74 ± 0.02 ^{aC}	1.59 ± 0.16 ^{bD}	1.07 ± 0.00 ^{cC}	11.5 ± 0.23 ^{aA}	3.37 ± 0.15 ^{bB}	2.07 ± 0.13 ^{cB}	5.67 ± 0.24 ^{aB}	2.54 ± 0.01 ^{bC}	1.63 ± 0.14 ^{cB}	11.5 ± 0.15 ^{aA}	4.68 ± 0.21 ^{bA}	4.30 ± 0.14 ^{bA}
Total Lignans	3.45 ^{aC}	1.59 ^{bD}	1.07 ^{cC}	15.87 ^{aA}	3.44 ^{bB}	2.08 ^{cB}	9.04 ^{aB}	2.56 ^{bC}	1.63 ^{bB}	17.03 ^{aA}	6.19 ^{bA}	4.30 ^{bA}
Other Phenolic Compounds												
Vanillin	n.d.	n.d.	n.d.	0.29 ± 0.04 ^{B}	n.d.	n.d.	0.55 ± 0.05 ^{A}	n.d.	n.d.	0.20 ± 0.03 ^{B}	n.d.	n.d.
Phloridzin	0.03 ± 0.01	n.d.	n.d.	n.d.	n.d.	n.d.	0.04 ± 0.00	n.d.	n.d.	n.d.	n.d.	n.d.
Total Phenolic Compounds	61.3 ^{aB}	4.38 ^{cD}	14.8 ^{bB}	85.2 ^{aB}	24.2 ^{bB}	14.1 ^{cB}	49.9 ^{aB}	10.8 ^{bC}	10.5 ^{bC}	635 ^{aA}	300 ^{bA}	329 ^{bA}
Non-Phenolic Compounds												
Gluconic acid	55.5 ± 7.45 ^{aB}	21.7 ± 3.83 ^{bC}	5.14 ± 0.24 ^{bC}	102 ± 10 ^{bA}	315 ± 13.0 ^{aB}	6.56 ± 0.59 ^{cC}	49.8 ± 4.90 ^{bB}	488 ± 44 ^{aA}	14.3 ± 1.01 ^{bA}	30.5 ± 2.18 ^{bB}	273 ± 29 ^{aB}	11.2 ± 0.55 ^{bB}
Fatty Acids												
Trihydroxyoctadecadienoic acid	2.06 ± 0.14 ^{aB}	0.54 ± 0.27 ^{bB}	0.02 ± 0.01 ^{bB}	1.84 ± 0.12 ^{aB}	0.76 ± 0.03 ^{bB}	0.10 ± 0.02 ^{cB}	1.15 ± 0.06 ^{aC}	0.20 ± 0.04 ^{bB}	0.02 ± 0.01 ^{bB}	3.76 ± 0.27 ^{aA}	4.07 ± 0.31 ^{aA}	1.80 ± 0.06 ^{bA}
Trihydroxy octadecenoic acid (pinellic acid)	3.78 ± 0.30 ^{aB}	0.22 ± 0.00 ^{bB}	0.28 ± 0.03 ^{bB}	4.51 ± 0.22 ^{aB}	0.60 ± 0.02 ^{bB}	0.16 ± 0.03 ^{bB}	56.0 ± 2.75 ^{aA}	12.8 ± 1.55 ^{bA}	7.22 ± 1.17 ^{bA}	4.90±0.32 ^{aB}	1.32 ± 0.05 ^{bB}	0.80 ± 0.01 ^{bB}
Octadecanedioic acid	0.17 ± 0.01 ^{B}	n.d.	n.d.	2.63 ± 0.16 ^{aB}	0.57 ± 0.01 ^{bB}	0.09 ± 0.01 ^{cB}	45.2 ± 1.72 ^{aA}	3.11 ± 0.33 ^{bA}	1.63 ± 0.25 ^{bA}	0.25 ± 0.00 ^{aB}	0.05 ± 0.01 ^{bB}	0.005 ± 0.005 ^{cB}
Hydroxyl octadecadienoic acid	0.05 ± 0.01 ^{C}	n.d.	n.d.	1.50 ± 0.13 ^{B}	0.08 ± 0.02^{B}	n.d.	10.3 ± 0.45 ^{aA}	4.49 ± 0.15 ^{bA}	0.16 ± 0.00 ^{c}	1.09 ± 0.07 ^{B}	0.20 ± 0.01^{B}	n.d.
Total Fatty Acids	6.05 ^{aC}	0.76 ^{bC}	0.30 ^{bB}	10.49 ^{aB}	2.00 ^{bC}	0.36 ^{bB}	112.71 ^{aA}	20.67 ^{bA}	9.03 ^{cA}	10.00 ^{aB}	5.63 ^{bB}	2.60 ^{cB}

n.d.—not detected; LOQ—limit of quantification; MeOH 50%—methanol 50%; NADES 1—glycerol:urea (1:1); NADES 2—citric acid:sorbitol (1:2). All analyses were conducted in duplicate and data are represented by the mean ± standard error. The results were analyzed using one-way analysis of variance (ANOVA) followed by Duncan's new multiple range test. Different letters in each row and for each compound mean significant differences ($p < 0.05$) among extracts: uppercase letters indicate significant differences between the four parts of the fruits, while a lowercase letter denotes significant differences between solvents.

Gluconic acid was identified in extracts derived from all fruit parts. The use of NADES 1 (Gly:U, 1:1) resulted in a significant improvement in the extraction of this component from seed-containing pulp, seeds, and peel. In the case of seeds, where this compound was found in higher amounts, NADES 1 increased the extraction of gluconic acid by more than 900% in comparison to methanol. Gluconic acid is currently employed in a number of industrial sectors, including construction (45%), food (35%), pharmaceuticals (10%), and others (10%) [43].

The composition of fruits is influenced by a number of factors, including the variety, the degree of ripeness, the geographical location, the growing conditions, the cultivar, the time of harvest, and the determination methods [44,45].

3.3. Antioxidant Activity of Extracts

One of the most renowned biological studies conducted on plant extracts with a positive impact on human health is the antioxidant activity. This biological activity is closely linked to the high content and diversity of secondary metabolites found in plants, mainly phenolic compounds [46,47], which can delay the establishment and improve the recovery of several oxidative-stress-associated diseases, such as cancer, neurodegenerative disorders, and cardiovascular diseases. It is not possible to accurately assess the antioxidant capacity of any plant extracts using a single assay due to their immense complexity as multicomponent mixtures [48]. For this reason, this study employed four distinct approaches, each displaying a different mechanism: single electron transfer (FRAP), atom hydrogen transfer (ORAC), and two combined mechanisms (DPPH and ABTS), in order to gain a deeper understanding of the extract's true antioxidant capabilities [49,50].

In the DPPH assay (Figure 2a), no significant differences were observed in the antioxidant activity of extracts derived from the various fruit parts, with the exception of seeds, and when different solvents were employed. The methanol 50% seed extracts exhibited a higher antioxidant activity than the NADES. The ABTS method revealed a significant variance in the antioxidant capacity of the different fruit parts and solvents used (the values ranged between 2.03 and 80.22 mg_{TE}/g_{DW}) (Figure 2b). The peel extract demonstrated the highest potential to scavenge the ABTS radical, with similar results obtained for the methanol 50% and NADES 1 (Gly:U, 1:1) extracts. Andreu et al. [46] observed a similar pattern in five out of the six studied cultivars of *O. ficus indica* from Spain using methanol 80% as the solvent. The extracts derived from the seed-containing pulp and seeds demonstrated comparable activity when NADES 1 and methanol were employed as solvents. For the pulp extracts, a significant difference was observed between the extraction solvents, with methanol 50% demonstrating the most promising activity. Regardless of the solvent employed, the seeds demonstrated the lowest capacity to scavenge the radical ABTS (2.04 to 6.89 mg_{TE}/g_{DW}). The pattern of FRAP (Figure 2c) is very similar to the ABTS (values ranging from 0.87to 100.23 mg_{AAE}/g_{DW}), yet in FRAP, the highest ability to reduce ferric ions was observed in the pulp methanol extract, followed by the peel methanol extract, which aligns with the findings of El Mannoubi et al. [32]. In the study, conducted on Tunisian red and yellow–orange *O. ficus indica* fruits, three different solvents were employed (methanol 80%, ethanol 80%, and acetone 80%). It was observed that all pulp extracts exhibited higher FRAP values than the peel extracts. The methanol 50% solvent was found to be the most effective, with the exception of the seeds, where NADES 1 (Gly:U, 1:1) demonstrated an equivalent antioxidant activity. The seed extracts exhibited the lowest activity in FRAP, as in ABTS assays. The highest result was obtained in the peel NADES 1 (Gly:U, 1:1) extract (174.18 ± 3.57 mg_{TE}/g_{DW}) using the ORAC methodology (Figure 2d), with no significant differences observed between this extract and methanol 50%. In accordance with findings of Gómez-Maqueo et al. [51], the methanolic peel extract demonstrated the highest ORAC activity in three varieties of *O. ficus indica*, in comparison to the pulp. Conversely, the findings for *Opuntia dillenii* from the Canary Islands were the opposite. Nevertheless, NADES 1 was identified as the optimal solvent for the extraction of compounds with the capacity to quench free radicals via hydrogen donation from the

seed-containing peel, in conjunction with NADES 2 from the seeds. In the case of the two fruit parts, NADES 2 demonstrated comparable results to methanol 50%. Significant differences were observed between the solvents for the pulp extracts, with methanol 50% being the most effective, followed by NADES 1.

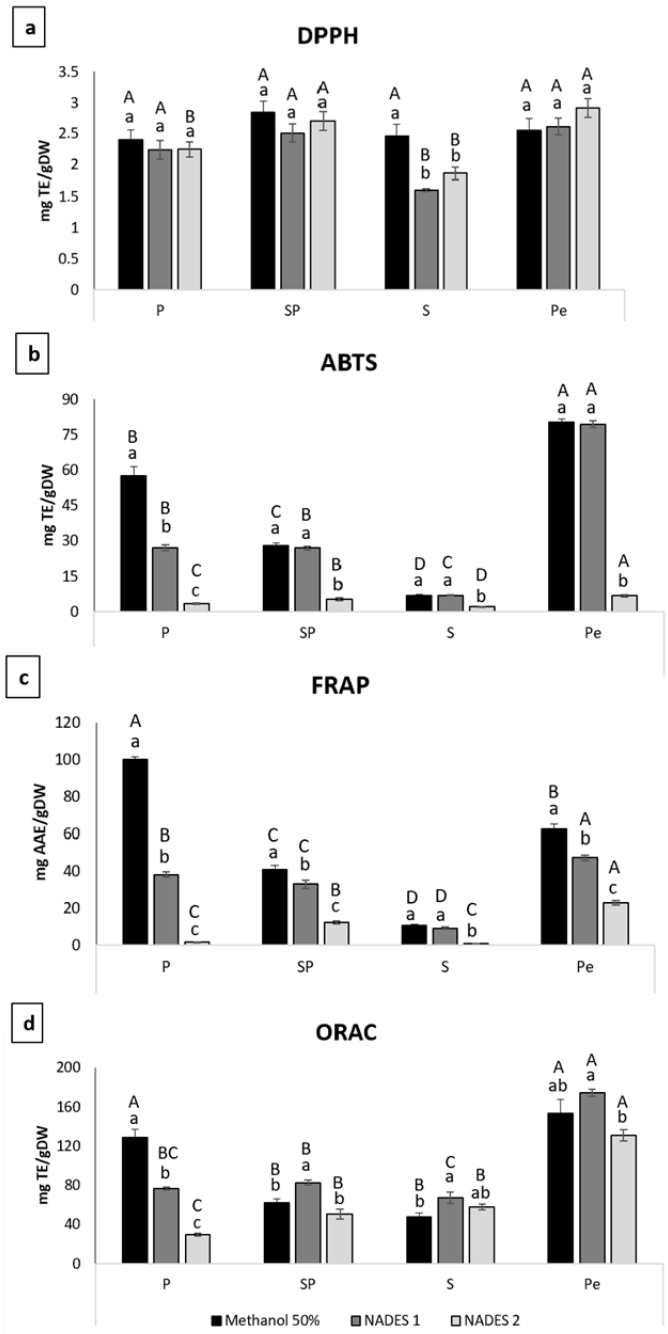

Figure 2. Antioxidant capacity, determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), ferric reducing antioxidant power (FRAP), and oxygen radical absorbance capacity (ORAC) assays, of extracts from different fruit parts (P, pulp; SP, seed-containing pulp; S, seeds; and Pe, peel) of *O. leucotricha* obtained with different extraction solvents (methanol 50%; NADES 1, glycerol:urea, 1:1; NADES 2, citric acid:sorbitol, 1:2). Values are expressed as mean ± SE. Different letters in each series indicate significant differences ($p < 0.05$) (Duncan's new multiple range test). Uppercase letters indicate significant differences ($p < 0.05$) between the four parts of the fruits, while a lowercase letter denotes significant differences ($p < 0.05$) between solvents.

The findings indicated that NADES 1 may offer a suitable alternative to conventional solvents for the extraction of antioxidants from the fruits of *O. leucotricha*.

3.4. Enzyme Inhibitory Capacity

Over the past few decades, there has been a growing interest in enzyme inhibitory agents derived from plants, with their potential for application in the treatment of various physiological disorders [52]. The current study evaluated the potential of the methanol extracts from the pulp, seed-containing pulp, seeds, and peel of *O. leucotricha* to inhibit a range of enzymes, including acetylcholinesterase (AChE), butyrylcholinesterase (BChE), tyrosinase, α-glucosidase, and α-amylase. This work reports the inhibitory activity on *O. leucotricha* extracts for the first time, but only of the methanol extract due to the inhibitory activity of the NADES itself.

The inhibition of the enzymes AChE (hydrolyzes acetylcholine) and BChE (metabolizes both acetylcholine and butyrylcholine) [53] represents a potential avenue for the treatment of Alzheimer's disease and Parkinson's disease, given that these neurodegenerative disorders are associated with the cholinergic deficit, which is characterized by a significant decrease in acetylcholine amount [54]. In the results of this study, as presented in Table 2, the seed extracts exhibited the highest inhibition of AChE (21.95 ± 1.68%), followed by the seed-containing pulp, peel, and pulp extracts, in comparison to the percentage inhibition of galanthamine (78%). Conversely, no significant variation was observed between the pulp, seed-containing pulp, and seed extracts in inhibiting BChE. The lowest activity was detected in the peel. With regard to BChE, galanthamine exhibited an inhibition of 40%. Tyrosinase (Tyr) is a glycosylated and copper-containing oxidase [55] that is employed as a depigmentation agent for human skin in the cosmetics and medical fields, as well as an anti-browning compound in the food and agriculture industries [56]. The results (Table 2) indicate that the greatest inhibition of this enzyme was achieved with seed extract (31.9 ± 2.17%) (the inhibition of Tyr by kojic acid was about 85%). With regard to the other fruit parts, no significant differences were observed. In contrast, Atiya et al. [57] reported that the methanol extracts from *O. ficus indica* fruit peel exhibited a greater tyrosinase inhibitory effect than pulp.

Table 2. Enzyme inhibitory capacity percentages (%) of methanolic extracts of the pulp, seed-containing pulp, seeds, and peel extracts from *O. leucotricha*.

Fruit Parts	Pulp	Seed-Containing Pulp	Seeds	Peel
Acetylcholinesterase	13.5 ± 0.5 ^{c}	18.42 ± 0.27 ^{b}	21.95 ± 1.68 ^{a}	15.22 ± 0.86 ^{bc}
Butyrylcholinesterase	20.67 ± 0.77 ^{a}	18.05 ± 1.39 ^{a}	22.3 ± 2.0 ^{a}	7.57 ± 0.2 ^{b}
Tyrosinase	24.1 ± 1.59 ^{b}	22.98 ± 1.77 ^{b}	31.9 ± 2.17 ^{a}	25.05 ± 2.0 ^{b}
α-Glucosidase	39.02 ± 2.12 ^{a}	40.53 ± 0.66 ^{a}	19.79 ± 1.77 ^{b}	35.71 ± 0.47 ^{a}
α-Amylase	44.75 ± 0.94 ^{a}	33.25 ± 2.93 ^{b}	34.37 ± 0.58 ^{b}	44.27 ± 1.14 ^{a}

% inhibitions are expressed as mean ± SE (n = 3). Different letters in each enzyme activity indicate significant differences ($p < 0.05$) (Duncan's new multiple range test) between the four parts of the fruits.

One of the approaches used to regulate blood glucose levels is the inhibition of α-glucosidase and α-amylase, which decrease the rate of blood sugar absorption and therefore treat diabetes mellitus (hyperglycemia) [58,59]. α-Glucosidase is the key intestinal enzyme in carbohydrate digestion [60], while α-amylase is responsible for postprandial glucose levels [61]. Table 2 indicates that seed-containing pulp, pulp, and peel extracts exhibited the highest inhibition values for α-glucosidase, with values ranging from 35 to 40%. These results were higher than those presented by Medina-Pérez et al. [62], in which an α-glucosidase inhibition of 24–30% was obtained with extracts (10 mg/mL) from different parts of *Opuntia oligacantha* fruit (endocarp, mesocarp, pericarp, and whole fruit). The pulp and peel extracts were also the most effective in inhibiting α-amylase, with an inhibition of 44%. This is comparable to the inhibition observed for *O. oligacantha* extracts at similar concentrations [62].

3.5. Pearson's Correlation and Principal Component Analysis (PCA)

The most relevant individual phenolic compounds from the pulp, the seed-containing pulp, seeds, and the peel extracts from *O. leucotricha* identified by HPLC-HRMS (piscidic acid, eucomic acid, dihydroferulic acid glucuronide isomer I, dihydroferulic acid glucuronide isomer II, sinapic acid, isorhamnetin rutinoside-rhamnoside, narcissin, naringenin, syringaresinol hexoside isomer I, syringaresinol hexoside isomer II), the total phenolic, flavonoid, and tannin contents, and the antioxidant activities were analyzed using Pearson's correlation and principal component analysis (PCA). A heatmap corresponding to Pearson's correlation coefficients was created (Figure 3).

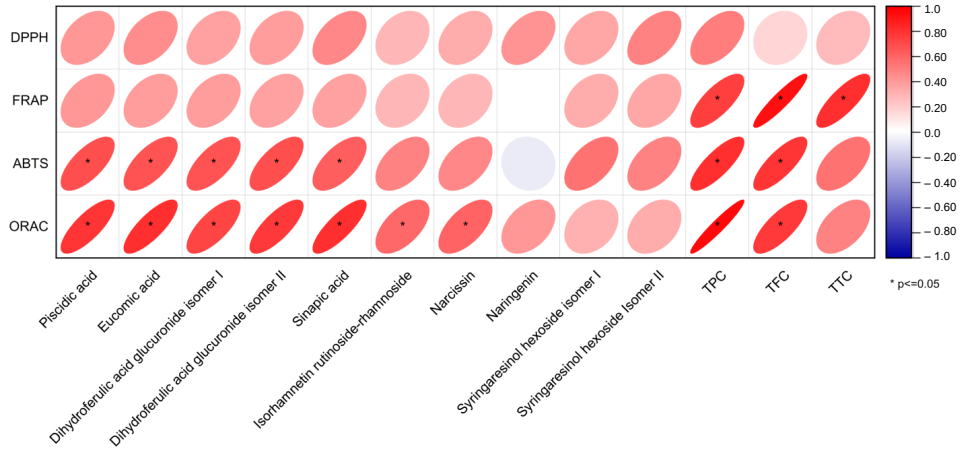

Figure 3. Heatmap corresponding to Pearson's correlation coefficients between the most relevant phenolic compounds identified by HPLC-HRMS, total phenolic compounds, total flavonoids, total tannins, and antioxidant capacities (DPPH, FRAP, ABTS, and ORAC) from the pulp, seed-containing pulp, seeds, and peel extracts from *O. leucotricha*. * Correlation is significant ($p \leq 0.05$). A more elliptical shape indicates a greater correlation.

A strong correlation was observed between TPC and the antioxidant activities (ABTS, FRAP, and ORAC assays) of the methanol and NADES extracts, as well as between the total flavonoid content (TFC) and the antioxidant activities, measured with the same assays, of the methanol and NADES extracts. Similar results were obtained in other *Opuntia* studies, namely on *Opuntia joconostle* [63] and *O. ficus indica* (green-skinned), *Opuntia streptacantha* (red-skinned), *O. stricta* (yellow-skinned), and *Opuntia lindheimeri* (purple-skinned) cactus pear [64], in which the authors noted that the ORAC was highly correlated with the TFC. Furthermore, study [65] on the peel, pulp, and seeds of wild prickly pear fruits (*Opuntia macrocentra*, *Opuntia phaeacantha*, and *O. engelmannii*) and commercial prickly pear fruits (*O. ficus indica*) demonstrated a positive correlation between the TFC and ABTS, and the TPC was positively correlated with the DPPH and FRAP assays. In regard to the TTC, only a strong correlation was observed with the FRAP assay. In the case of the individual phenolic compounds, a very strong correlation was achieved between piscidic acid, eucomic acid, dihydroferulic acid glucuronide isomer I, dihydroferulic acid glucuronide isomer II, and sinapic acid and the ORAC and ABTS assays. Furthermore, a moderate correlation was observed between the ORAC methodology and isorhamnetin rutinoside-rhamnoside and narcissin. The results demonstrate that these individual compounds are the primary contributors to the antioxidant capacity of the *O. leucotricha* extracts. Additionally, the findings suggest that the NADESs tested in this study are effective extractants of phenolics from this plant species.

The results for the most relevant individual phenolic compounds identified by HPLC-HRMS, the total phenolic compounds, total flavonoids, total tannins, and antioxidant capacities (DPPH, FRAP, ABTS, and ORAC), were employed to examine similarities and differences between different fruit parts and between different extracting solvents. This was achieved through the use of a biplot principal component analysis (PCA) (Figure 4).

The extracts from each tested solvent and each fruit part are represented by red points, while the contributions of each variable to the score are represented by the blue color. The overall variance in the dataset is 79.7%, and it was described by the first two principal components (PCs), of which the first (PC1) accounted for 62.23% and the second (PC2) 17.47% of the data variability in the dataset. The PCA biplot demonstrates a clear separation of the peel extracts obtained using methanol 50%, NADES 1, and NADES 2 from all other fruit parts' extracts. They are situated within the positive PC1 axis and are distinguished by elevated values across all tested components, a finding that is consistent with the results presented in reference [66]. The results that can be observed in the graph indicate that there was a greater differentiation between the use of the organic solvent and the use of NADES in extracts from the peel and pulp. In contrast, the seed extracts (either seed alone or seed plus pulp) exhibited a slight differentiation, with the extracting power of both NADES being highly similar. Moreover, the results presented in the loading plot indicate that there is minimal differentiation between the most relevant individual phenolic compounds identified by HPLC-HRMS and another association between total phenolic compounds, total flavonoids, and total tannins and antioxidant capacities (FRAP, ABTS, and ORAC). However, and a slight differentiation is evident for DPPH. In contrast, the pulp extracts obtained with methanol, followed by NADES 1, exhibit an excellent antioxidant capacity. In both peel and pulp extracts rich in bioactive compounds, a clear differentiation can be observed according to the solvent used in the extraction. In the case of the pulp, NADES 1 represents a viable alternative to the organic solvent. Conversely, in the case of the peel, both NADES 1 and 2 may be considered as potential options, contingent on the desired composition. Consequently, NADES 2 peel extracts exhibited higher flavonoid and cinnamic acid contents (see also Table 2), whereas NADES 1 presented compounds with the highest antioxidant capacity, as determined by ABTS, FRAP, and ORAC assays (Figure 2b–d). Finally, the extracts obtained with seeds (pulp + seed or simply seed) have a composition that is very similar, with the solvent used having a similar effect on the extraction of the bioactive components studied.

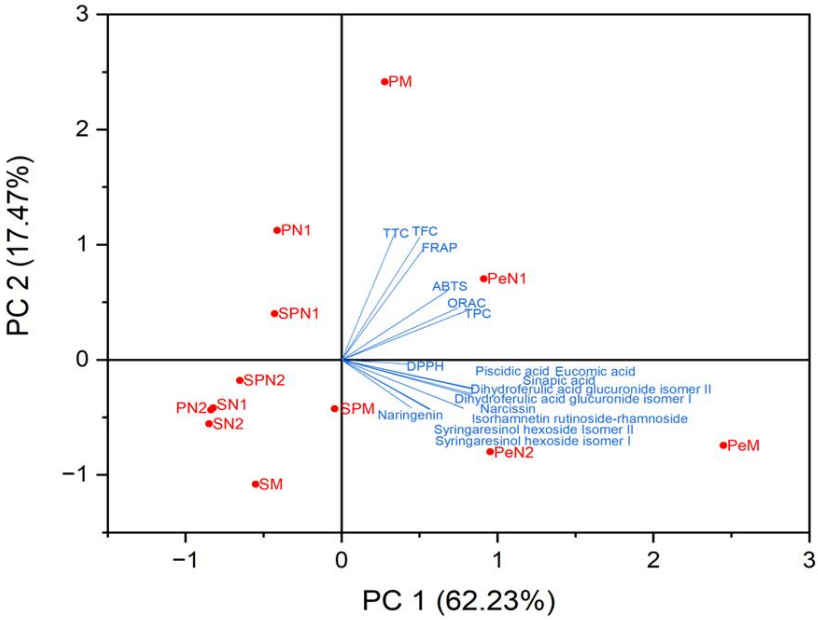

Figure 4. Biplot principal component analysis (PCA) of extracts from *O. leucotricha* fruit parts using NADES and a conventional solvent (methanol 50%). PM: methanolic extract of the pulp, PN1: NADES 1 extract of the pulp, PN2: NADES 2 extract of the pulp, SPM: methanolic extract of the seed-containing pulp, SPN1: NADES 1 extract of the seed-containing pulp, SPN2: NADES 2 extract of the seed-containing pulp, SM: methanolic extract of the seeds, SN1: NADES 1 extract of the seeds, SN2: NADES 2 extract of the seeds, PeM: methanolic extract of the peel, PeN1: NADES 1 extract of the peel, PeN2: NADES 2 extract of the peel.

4. Conclusions

This study aimed to investigate the chemical profile, antioxidant capacities, and enzyme inhibitory activities of the pulp, seed-containing pulp, seeds, and peel extracts from *Opuntia leucotricha* DC. We employed two different solvent systems: NADES glycerol:urea (1:1) and citric acid:sorbitol (1:2), in comparison with the conventional solvent methanol 50%. The peel extract exhibited the highest content of bioactive compounds and bioactivity, followed by the pulp+seed extract, the pulp extract, and the seed extract. The peel extracts exhibited high contents of all the families of compounds studied, with a notable presence of phenolic acids, which were also observed in the pulp extracts. Seed extracts, on the other hand, were characterized by the presence of lignans and other components. In general, the organic solvent demonstrated the highest efficiency in extracting the bioactive compounds and antioxidant capacities of the different fruit parts of *O. leucotricha*. This was followed closely by NADES 1 (Gly:U, 1:1). Notably, this green solvent is an excellent extractor of the abundant non-phenolic compound gluconic acid, especially in seeds. The methanol extracts also demonstrated significant inhibitory enzymatic activities, particularly with regard to tyrosinase. The results obtained suggest the extracts from different parts of *O. leucotricha* fruits as an interesting source of compounds with antioxidant and enzyme inhibitory compounds for food and industrial applications. Moreover, these findings encourage the use of NADES as a promising sustainable, effective, and greener alternative to traditional organic solvents for future extractions of *Opuntia* spp.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/horticulturae10080824/s1, Table S1: HPLC-HRMS characteristics of identified compounds in extracts [Methanol 50%, NADES 1 (glycerol:urea, 1:1), and NADES 2 (citric acid:sorbitol, 1:2)] of different parts (pulp, seed-containing pulp, peel, and seeds) from *Opuntia leucotricha* DC.; Table S2. Summary of HPLC-HRMS criterion for quantification of phenolics in *Opuntia leucotricha* DC. extracts; Figure S1: HPLC-HRMS extracted-ion chromatogram of compounds identified and quantified in *Opuntia leucotricha* DC. fruit extracts.

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