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Chemical Composition, Nutritional Value, Antioxidative, and *In Vivo* Anti-inflammatory Activities of *Opuntia Stricta* Cladode

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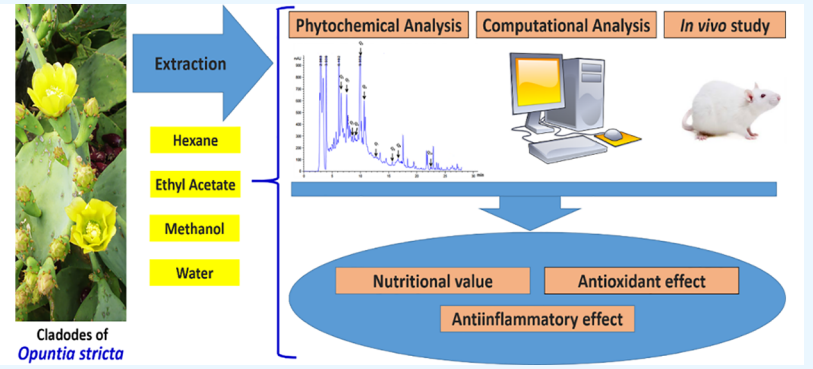

ABSTRACT: The cactus family plant has been used in folk medicine for a long time. In this work, *Opuntia stricta* chemical composition and its antioxidative and anti-inflammatory properties were investigated. Our results showed that *O. stricta* is highly rich in fibers and minerals. The present study assessed the levels of polyphenol contents and antioxidant and in vivo anti-inflammatory activities. The highest phenolic compounds and antioxidant activity were observed in the methanolic extract. Concerning the qualitative analysis, nine phenolic and organic acids were identified and quantified by high-performance liquid chromatography (HPLC). Luteolin-7-Glu (4.25 μg/g), apigenin-7-Glu (3.15 μg/g), and catechin (2.85 μg/g) were identified as major phenolic compounds. The predominant fatty acids detected by gas chromatography (GC) coupled to a flame ionization detector were linoleic and linolenic acids (35.11%). A factorial design plan was used to determine the effect of temperature, agitation speed, and maceration period on phenolic contents. *In vivo*, the methanol extract from *Opuntia stricta* showed anti-inflammatory activity. The computational modeling reveals that *O. stricta* compounds bind VEGF, IL-6, and TNF-α with high binding scores that reach −8.7 kcal/mol and establish significant molecular interactions with some key residues that satisfactorily explain both *in vitro* and *in vivo* findings. These data indicate that *Opuntia stricta* cladode powder could be potentially useful in pharmaceutical and food applications.

INTRODUCTION

Owing to their richness in biologically active substances having different structures, medicinal plants are considered as an important source for pharmaceutical molecules.[1−4] Obviously, they contain great amounts of phenols, coumarins, alkaloids, acids, tannins, lignanes, terpenes, and flavonoids, which have multiple interests in nutrition, industry, and pharmacy.[1,3] Several studies have highlighted that herb extracts exert an antioxidant effect, a peculiarly sought-after property in treating and preventing different pathologies.[4−6] Fibers contained in plant-based foods reduce the risk of the development of many diseases, such as cancer, cardiovascular, and neurodegenerative ones.[1,4,6] Recently, great consensus has been reached on the preventive/curative role of vegetal products, such as fruits and oils seeds, because of their antioxidant potential.[5,7]

Cactaceae plants have been widely used in folk medicine and ethnopharmaceutical applications.[1,7] Among this family, the *Opuntia* genus gathers more than 200 different species that mainly grow in arid and semiarid areas.[7,8] Thanks to their chemical compositions, medicinal plants and nutraceuticals endow antioxidant, antinociceptive, and anticancer effects.[7,9,10] Young cladodes of *Opuntia*, also known as nopalitos, are consumed as vegetables and contain many antioxidant compounds, such as polyphenols, flavonoids, beta-cyanin, and

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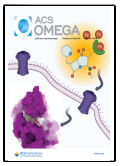

beta-xanthin.[7] Cladodes of *Opuntia* serve to treat many pathologies, such as burns, edema, inflammation, diabetes, gastrointestinal problems, and viral infections.[1,11,12] The phytochemical composition revealed a promising total composition of phenolics, flavonoids, tanins, and proanthocyanidins using acetone, water, and ethanol as solvents. The composition revealed rich content of vitamins, especially vitamins A, E, and C.[7,13,14] In this context, the current work aims to evaluate some biological activities of extracts from nopalitos of *O. stricta*. Additionally, the binding affinities and molecular interactions of *O. stricta*-identified compounds with VEGF, IL-6, and TNF-α receptors were explored by using computational analyses.

MATERIALS AND METHODS

Plant Material. Fresh cladodes of *Opuntia stricta* were collected from the district of Sidi Bouzid (Tunisia) (latitude 35° 2′ 25″N, longitude 9° 29′ 37′′ E; elevation: 41 m) in February 2021. The plant was assigned a Voucher specimen number, OS1 at the herbarium of the Faculty of Sciences, Sfax University, Tunisia. After spine removal, the cladodes were washed, cut into small pieces, and dried at dark room temperature. The obtained dried material was then finely ground to obtain a smooth powder.

Extraction's Procedure. The cladode powder was macerated (1:20 w/v) with continual stirring for 72 h using either hexane (Hex), ethyl acetate, methanol, or water as solvents. The recovered macerates were, thereafter, filtrated, and solvents were evaporated in a rotary evaporator (Rotavapor R210, Buchi, Switzerland) at 40 °C. The extracts were stored in a dark polyvinyl container at 4 °C until further analyses.

Phytochemical Characterization of *O. Stricta* Extracts.

Nutritional Value of the Opuntia Stricta Cladode. The crude fine powder was used to evaluate the nutraceutical potential of *O. stricta*. Protein and dry matter were estimated according to the AOAC methods.[15] Fat content was determined by Soxhlet extraction with hexane for 6 h at 69 °C. The content of total dietary fiber was measured. Following incineration in a muffle furnace at 550 °C for 2 h, the obtained ash from the cladode powder was digested using HNO_3 and dissolved in deionized water.[16] The major minerals contained in the obtained solution were measured by flame atomic absorption spectrometry (Hitachi Z-6100, Japan).

Assessment of the Total Phenolic Content. The total phenolic content was assessed by Folin−Ciocalteu's method.[17] Briefly, 200 μL of the sample or gallic acid was added to 2.6 mL of distilled deionized water. 200 μL of Folin−Ciocalteu's phenol reagent was added and mixed. After 6 min, 2 mL of a Na_2CO_3 solution (7%, w/v) was added and mixed. After incubation for 90 min, absorbance was measured at 750 nm. The total phenolic content was measured from a standard curve using gallic acid as the probe, and expressed as mg of gallic acid equivalent (GAE)/g of dry weight.[18] All samples were collected in triplicate.

Total Flavonoids Content. The total flavonoid content was assessed according to the method described by Zhishen et al. (1999).[19] A sample of each extract was mixed with 120 μL of 5% sodium nitrite. After 5 min of incubation, 120 μL of a 10% aluminum chloride solution was added. Six minutes later, 800 μL of 1 M sodium hydroxide solution was added to the mixture. The absorbance was determined to be 510 nm. Catechin was used as a probe, and total flavonoid contents were expressed as milligrams of catechin equivalents/g of dry weight (mg/g CE).

Measurement of Condensed Tannins. The amount of condensed tannins was measured by the vanillin method, as described by Broadhurst and Jones (1978).[20] Briefly, 3 mL of vanillin (4%) was added to 0.5 mL of each extract. Then, 1.5 mL of concentrated HCl was added. After incubation, absorbance was read at 500 nm in the dark at 20 °Cfor 15 min. The calibration curve was prepared by using solutions of different concentrations of catechin. Results are expressed in mg of catechin equivalent/g of dry weight (mg of CE/g of DW).

Measurement of Anthocyanin Content. The monomeric anthocyanin content of *Opuntia stricta* was determined spectrophotometrically using a pH-differential protocol as described by Broadhurst and Jones (1978).[20] Extracts were mixed thoroughly with 0.025 M potassium chloride buffer (pH 1) or sodium acetate buffer (pH = 4.5) at a ratio of 1:9 (v/v). The absorbance was measured at 510 and 700 nm, and the anthocyanin content was measured as follows:

$$\text{Total monomeric anthocyanins (mg/kg)} = \text{AbsxMWx1000}/(\varepsilon \text{xC}) \quad (1)$$

where Abs is the absorbance = $(A_{515} - A_{700})_{\text{pH 1.0}} - (A_{515} - A_{700})_{\text{pH 4.5}}$; MW is the molecular weight for cyanidin 3-glucoside = 449.2; ε is the molecular absorptivity of cyanidin 3-glucoside = 26900; and *C* is the concentration of the buffer in milligrams per milliliter.

HPLC Analysis. The identification of polyphenols contained in the methanolic extract (1 mg/20 mL) of cladodes was analyzed by HPLC using the 1100 series HPLC system with a diode array detector coupled to a computer (HP ChemStation) and a thermostat control. The identification was determined according to the method of Selvaggini et al. (2006).[21] The analysis was made using a C18 Nucleodur column (particle size 5 μm, L = 250 mm, d = 4, 6 mm) maintained at a temperature of 30 °C and a flow rate of 1.0 mL/min. The injected volume was 20 μL. Results are expressed in μg of phenols/g of dry weight.

Fatty Acids Composition. Fatty acid composition was assessed by gas chromatography (GC) coupled to a flame ionization detector following esterification with 2 M KOH to fatty acid methyl esters (FAMEs) according to the IUPAC standard method.[22] Analytical GC was carried out on a Hewlett−Packard 6890 gas chromatograph series II (Agilent Technologies, Palo Alto, California, USA) equipped with a VARIAN CP-SIL88 (50 m × 0.25 mm, 0.25 mm film thickness) capillary column. Initially, the temperature of the oven was held for 13.5 min at 175 °C, and then, ramped at 2 °C/min up to 185 °C and held isothermal for 3 min. Injector and FID detector temperatures were held at 220 and 280 °C, respectively. Identification of FAMEs was recorded as compared to the retention time of pure standards under similar conditions. The quantification of FAMEs was proportional to the percentage area of the lipid fraction.

***In Vitro* Determination of the Antioxidant Activity.**

DPPH Assay. The free scavenging activity of different extracts on the α-diphenyl-bpicrylhydrazyl ($DPPH^{\bullet}$) radical was determined according to Ozturk et al. (2011).[23] Overall, a mixture of 4 mL of methanol solution of DPPH (0.1 mM) and 1 mL of the extract solution (methanol, *n*-hexane, water, and ethyl acetate) at different concentrations (0−0.4 mg/mL) was used and kept in a dark room for 30 min of incubation. The free radical scavenging ability was assessed by determining the absorbance at 515 nm. The percentage inhibition of DPPH radicals was calculated as

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$$\text{Inhibition (\%) of DPPH radicals} = [(A_{control} - A_{test})/A_{control}] \times 100 \quad (2)$$

where $A_{control}$ is the absorbance of the control reaction, which contains all reagents except the plant extract, and A_{test} is the absorbance of the plant extract.

ABTS Assay. Antioxidant activity of *Opuntia stricta* was also analyzed by determining its ability to scavenge $ABTS^{\bullet+}$ free radicals, as reported by Ozgen et al. (2006).[24] Reaction mixtures containing 3 mL of reagent and 20 μL of sample were incubated in a water bath at 30 °C for 30 min, and then absorbance was measured at 734 nm. In fact, unpaired electrons were sequestered by antioxidants in the sample, and the test solution turned to a less intense color. The ABTS results were expressed as mM Trolox equivalents (TE) per gram of dry weight.

Superoxide Anion ($O_2^{\bullet-}$) Test. Superoxide radicals ($O_2^{\bullet-}$) were assessed as reported by Boulanouar et al. (2013).[25] Briefly, a nitro blue tetrazolium (NBT), methionine, and riboflavin photochemically reactive solution was prepared under 25 W fluorescent lamp lighting and mixed with 100 μg/mL of the extract. The photochemically reduced riboflavins generate $O_2^{\bullet-}$, which in turn reduces NBT to form blue formazan. The intensity of the formed blue color was recorded at 560 nm. The decrease of the blue intensity relative to the blank solution reflects the potential of the extract to chelate the generated $O_2^{\bullet-}$ radicals. The scavenging degree was calculated as follow:

$$\text{Scavenging (\%)} = [(A_{control} - A_{test})/A_{control}] \times 100 \quad (3)$$

where $A_{control}$ and A_{test} are the net values of absorbance measured for the control and test samples, respectively.

Hydroxyl ($OH^{\bullet}$) Radical-Scavenging Activity (HRSA). The scavenging activity of *Opuntia stricta* extracts on the hydroxyl radical ($OH^{\bullet}$) was determined by the deoxyribose method.[26] The reaction mixtures (10 mM phosphate buffer (pH = 7.4), 2.8 mM deoxyribose, 2.8 mM H_2O_2, 100 μM EDTA, 25 μM $FeCl_3$, and the test sample (200 μg)) were supplemented with ascorbic acid to a final concentration of 100 μM and then incubated at 37 °C for 1 h. The color was developed following the addition of 1% thiobarbituric acid and ice-cold trichloroacetic acid (2.8%) and heating in a boiling water bath (95−100 °C) for 20 min. After cooling, the chromophore was extracted into n-butanol. Its absorbance was measured at 532 nm against n-butanol (as a blank). The reaction mixture without the sample was used for a comparison. The hydroxyl radical-scavenging activity (HRSA) was expressed as

$$\text{HRSA\%} = [1 - (A \text{ at 532 nm in presence of sample} / A \text{ at 532 nm in presence of sample})] \times 100 \quad (4)$$

Scavenging Activity Against Hydrogen Peroxide. The scavenging capacity of *Opuntia stricta* extracts on H_2O_2 was assessed as reported by Ruch et al. (1989).[27] While the tested tubes contained 2 mL of various plant extracts and 1.2 mL of H_2O_2 (40 mM), the blank mixture was prepared, similarly except for H_2O_2. After 10 min of incubation, the absorbance was determined to be 230 nm. The following formula was used to calculate the scavenging activity:

$$\text{\%scavenging activity} = [(A_{control} - A_{test})/A_{control}] \times 100 \quad (5)$$

where $A_{control}$ is the absorbance of the control and A_{test} is the absorbance of the extract.

Nitric Oxide Radical Scavenging Activity. Nitric oxide radical scavenging activity of *O. stricta* was determined using the method described by Marcocci et al. (1994).[28] The reaction mixture containing sodium nitroprusside (2 mL, 10 mM in 0.5 M phosphate buffer, pH 7.4) and 250 μL of extracts at different concentrations was incubated at 25 °C for 150 min. 0.5 mL of the reaction mixture was added to a test tube containing sulfanilamide (1 mL, 1% in 5% phosphoric acid) and incubated at 25 °C for 5 min. Then, 1 mL of 0.1% (1-naphthyl)-ethylenediamine was added to the solution and left for 30 min at 25 °C. The absorbance of the final solution was assessed at 546 nm. The percentage of nitric oxide radical scavenging was determined using the formula

$$\text{\%inhibition} = [(A_{control} - A_{test})/A_{control}] \times 100 \quad (6)$$

where $A_{control}$ is the absorbance of the control reaction and A_{test} is the absorbance of the extract reaction.

Anti-inflammatory Activity of *O. Stricta* Extract. Adult *Wistar* rats, weighing 180 ± 20 g, were used to evaluate the anti-inflammatory activity of *O. stricta* extract. They were obtained from the regional Central Pharmacy, Tunisia and reared at standard conditions (temperature: 20 ± 2 °C; humidity: 60 ± 5%; and 12 h dark/light cycle). All rats received tap water and standard food pellets *ad libitum*. After 2 weeks of acclimation to the breeding house conditions, the experiment was conducted in accordance with the guide for the care and use of laboratory animals issued by the University of Sfax (Tunisia) and approved by its local Ethical Committee. Acute inflammation was induced by intraplantar injection of 100 μL of carrageenan solution (1% dissolved in saline physiologic solution at 0.9% NaCl).[4,29] Animals were distributed into three groups of six rats each. They were treated by intraperitoneal injection of saline physiologic solution (CARR group), indomethacin (at a dose of 10 mg/kg) (CAR+IND group), or the methanolic extract of *O. stricta* dissolved in saline physiologic solution (at a dose of 100 mg/kg) (CAR+OS group). The injected volume of treatments was kept constant (1 mL/kg).

The volume of the hindpaw edema was measured at 1, 2, 3, 4, and 5 h after the treatment, and the inflammatory response was evaluated as the percentage of variation of the edema volume relative to the initial status at each time point, according to the following formula

$$\text{\%edema} = [(P_T - P_0)/P_0] \times 100 \quad (7)$$

where P_T is the edema volume at 1, 2, 3, 4, or 5 h and P_0 is the edema volume immediately after the carrageenan injection.

After being euthanized by profound diethyl ether exposure, some specimens of the inflamed skin were dissected out and fixed in 4% formalin for 48 h. The skin specimens were embedded in paraffin, and then 5 μm thick sections were realized, prepared, and stained using hematoxylin- eosin staining for microscopic examination. The study procedure was approved by the local ethical committee of the medicine faculty of Sfax.

Computational Analysis and Interactions Assay. The *Opuntia stricta* compounds, which had been identified, were used in the computational study to assess their molecular interactions and confirm their potential biological activities. The 13 chemical structures of *O. stricta* phytochemicals were retrieved from the Pubchem Web site. The 3D crystal structures of VEGF, IL-6, and TNF-α receptors were obtained from the RCSB PDB. The phytochemicals and three targeted receptors were prepared, processed for minimization, and then saved in

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pdbqt format.[4,7,30] They were subjected to a CHARMm force field, as previously reported, after targeting the grid box by selecting some key residues within the pocket region.[7,30,31] VEGF, IL-6, and TNF-α receptors have been selected as they are commonly involved in anti-inflammatory and healing pathways.

Statistics. All *in vitro* studies were performed in triplicate. Statistics were carried out using SPSS for Windows software (version 20) (IBM Corporation). Data were expressed as the mean ± standard deviation (SD) and analyzed by one-way ANOVA followed by Duncan's multiple range test. The significance level was set at $p \leq 0.05$.

RESULTS AND DISCUSSION

Herbs are the main source of nutrients and constitute a natural scaffold for drugs discovery and development.[1,6,7,30,31] They provide the major metabolic substrates (proteins, carbohydrates, fat, vitamins, etc.) needed to sustain survival, growth, and functions of living organisms.[4,7] Because of their richness in biologically active molecules that improve the immune system and prevent or heal several pathologies and health disorders, various medicinal plants are considered by the WHO in several health care systems as functional foods and dietetic supplements.[1,7,30] In this work, we focused on evaluating the nutraceutical and pharmacological potential of *Opuntia stricta* (order of Caryophyllales, Family of Cactaceae). This plant has been grown in the Mediterranean region and served as food and forage.[7,29]

Table 1. Fatty Acid (FA) Composition (%) of *Opuntia Stricta*[a]

CX:Y	FA	RT	percent (%)
C10:0	capric acid	4.151	2.54 ± 0.18
C12:0	lauric acid	5.449	2.61 ± 0.22
C16:0	palmitic acid	7.722	20.76 ± 0.46
C14:0	myristic acid	6.245	0.20 ± 0.07
C18:0	stearic acid	11.091	11.84 ± 1.87
C20:0	arachidic acid	15.680	7.35 ± 1.47
C22:0	behenic acid	19.410	0.40 ± 0.20
C24:0	lignoceric acid	22.355	0.98 ± 0.82
Σ SFA	-	-	46.68 ± 1.12
C12:1	dodecenoic acid	5.977	0.60 ± 0.30
C14:1	myristoleic acid	6.407	0.33 ± 0.11
C16:1 w7	palmitoleic acid	8.809	0.77 ± 0.03
C16:1 w9	palmitoleic acid	8.452	1.49 ± 0.39
C17:1	heptadecenoic acid	9.595	0.72 ± 0.26
C18:1 w7	vaccenic acid	11.590	12.47 ± 7.87
C18:1 w9	oleic acid	11.636	1.12 ± 0.57
C20:1	eicosenoic acid	19.746	0.72 ± 0.46
Σ MUFA	-	-	18.22 ± 0.45
C18:2	linoleic acid	12.539	20.38 ± 1.70
C18:3	linolenic acid	13.985	14.73 ± 2.34
Σ PUFA	-	-	35.11 ± 0.62

[a]Values are mean of three triplicate determinations.

Phytochemical and Nutritive Profiling of the *O. Stricta* Extract. Our results revealed that *O. stricta* is highly enriched in nutritive elements (proteins, fat, fibers, and minerals). In particular, 100 g of *O. stricta* cladode contains 3.60 ± 0.33 and 2.97 ± 0.32 g of proteins and fats, respectively. It also comprises important amounts of minerals such as magnesium (0.85 ± 0.001 mg), manganese (2.90 ± 0.064 mg), copper (0.72 ± 0.020 mg), and zinc (1.83 ± 0.012 mg) (Table 1). Some minerals have been found in *Opuntia* in crystallized forms[37]. Iron, magnesium, zinc, manganese, copper, and many other minerals almost all have prominent functional elements and act mainly as enzymatic cofactors. For example, iron is a cofactor for hemoglobin transporting respiratory gases and is required as a supplement to treat anemia.[32] Copper, zinc, and manganese are essential for metalloenzymes' functions, such as superoxide dismutase (SOD) and catalase (CAT), which regulate oxidative stress.[4,30,31,33] In general, these minerals ensure electrolyte balance and enhance immune defense, muscles, and nervous system function. They are almost prescribed as supplements to treat hundreds of health disorders, particularly in a deficient diet. The average of extraction yields were 76.1 mg/g, 54.7 mg/g, 56.6 mg/g, and 80.8 mg/g for each of hexane, ethyl acetate, methanol (80%), and water, respectively.

Table 2. Major Components and Mineral Contents of *Opuntia Stricta* Cladode Powder[a,b]

parameter	cladode powder
dry matter (%)	89.38 ± 15.12
ash (%)	0.80 ± 0.09
protien (g of protein/100 g)	3.60 ± 0.33
fat (%)	2.97 ± 0.32
total fiber (g/100g)	54.02 ± 3.41
minerals *(mg/100g)*	-
Ca	2908.51 ± 26.16
K	43.92 ± 0.40
Mg	0.85 ± 0.001
Na	291.25 ± 1.25
Fe	6.90 ± 0.13
Zn	1.83 ± 0.012
Cu	0.72 ± 0.020
Mn	2.90 ± 0.064
Cr	0.90 ± 1.10
Ni	<0.01

[a]Values are means ± SD, n = 3. FA: fatty acid; SFA: saturated fatty acid; MUFA: monounsaturated fatty acid. [b]PUFA: polyunsaturated fatty acid.

Analysis by gas chromatography showed the presence of 18 fatty acids (8 SFAs, 8 MUFAs, and 2 PUFAs), in nopalitos with a higher percentage of SFAs (46.68 ± 1.12%). The most represented fatty acids in *O. stricta* cladodes are palmitic acid (20.76 ± 0.46%), stearic acid (11.84 ± 1.87%), vaccenic acid (12.47 ± 7.87%), linoleic acid (20.38 ± 1.70%), and linolenic acid (14.73 ± 2.34%) (Table 2). The fatty acid profile was similar to those commonly found in the *Opuntia* genus. Some FAs, such as stearic acid, have been proposed to replace dietetic solid fats in order to reduce the risk of cardiovascular diseases and cholestasis-induced liver disorder. It was also reported to prevent the oxidative stress-induced brain injury.[34−36] While stearic acid enhances the production of proinflammatory factors, the presence of palmitic, oleic, and linoleic in *O. stricta* reverts the inflammatory condition.[37] Linoleic acid also prevented cancer, coronary heart disease, autoimmune disorder, hypertension, and dyslipidemia.

In general, the supply of PUFA might reestablish their loss because of their oxidation in response to abiotic stress. In addition to components with a metabolic role, *O. stricta* is enriched in fiber (54.02 ± 3.41 g/100 g). Fibers are functional nutrients that prevent the development and progression of several chronic diseases.[38] In addition to its rich composition in nutrients, our study highlights the presence of great amounts of

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Table 3. Total Phenols, Flavonoid Contents, Anthocyanins, Condensed Tannins, Proteins, and Carbohydrate Content for the Studied Plant[i]

	phenolic compounds					
extracts	total phenols (mg GAE/g DW)	condensed tannins (mg CE/g DW)	total flavonoids (mg CE/g DW)	anthocyanins (mg/g DW)	carbohydrates (mg/mL)	proteins (mg/mL)
hexane	6.15 ± 1.06[a]	4.24 ± 0.5[a]	0.65 ± 0.01[a]	5.18 ± 1.1[a]	407.48 ± 18.61[c]	0.41 ± 0.32[a]
ethyl acetate	8.35 ± 1.4[d]	5.61 ± 1.01[b]	4.59 ± 1.01[b]	15.03 ± 3.02[c]	387.70 ± 21.12[b]	0.70 ± 5.42[a]
methanol (80%)	12.63 ± 2.3[c]	7.38 ± 2.2[c]	6.92 ± 1.80[d]	18.16 ± 5.1[d]	315.50 ± 15.41[a]	10.38 ± 0.78[c]
water	7.93 ± 1.9[b]	4.50 ± 0.9[a]	5.13 ± 1.50[c]	7.10 ± 2.03[b]	294.97 ± 23.66[b]	5.28 ± 1.01[b]

[i]Data are presented as mean ± SD of three individual determinations; GAE = gallic acid equivalents; CE = catechin equivalents; DW = dry weight; values having different letters on a same column showed significant difference ($p < 0.05$). The results are sorted in increasing order: a < b < c < d.

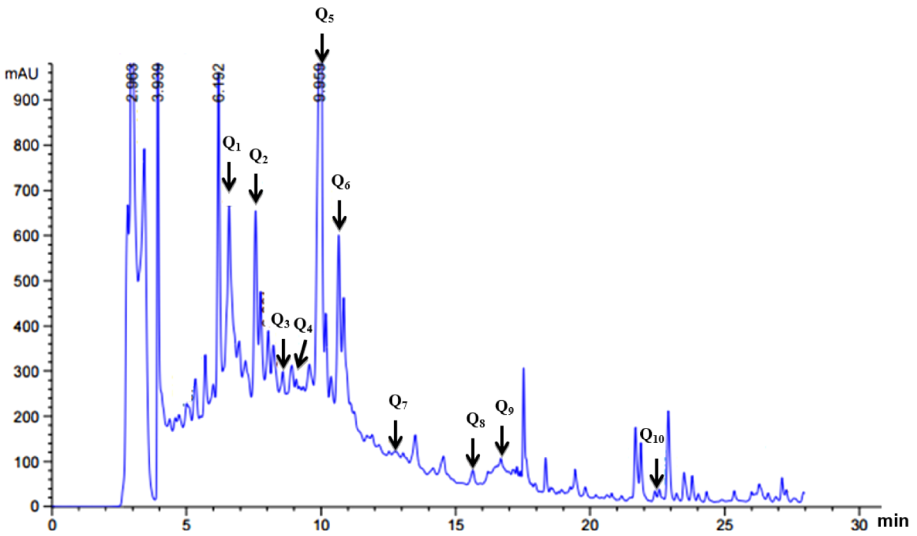

Figure 1. HPLC chromatogram of *O. stricta*.

Table 4. Chemical Composition of Methanol Extracts from the *Opuntia stricta* Powder Cladode by HPLC[a]

short name	retention time (min)	composition (μg/g)
(1) catechin	6.58	2.85
(2) tyrosol	6.95	nd
(3) 4HO-benz	7.56	1.54
(4) rutin	8.72	0.47
(5) verbascoside	8.90	1.21
(6) luteolin-7-glu	9.33	4.25
(7) apigenin-7-glu	10.83	3.15
(8) oleuropein	11.70	0.74
(9) quercetin	15.21	0.19
(10) pinoresinol	15.90	0.86
(11) cinnamic	16.52	nd
(12) apigenin	16.95	nd
(13) ellagic acid	22.56	0.08

[a]nd: not detected.

phenolic compounds in different solvent extracts. In particular, the methanolic extraction of *O. stricta* cladode yields greater amounts of total phenols (12.63 ± 2.3 mg GAE/gDW), flavonoids (6.92 ± 1.80 mg CE/g DW), and anthocyanins (18.16 ± 5.1 mg/g DW) than other solvents (Table 3, Figure S1). The quantity of total phenols in the cladode is much higher than that in fruits, as reported in other studies.[7,39] Such an influence of the solvent's polarity on the extraction of these bioactive compounds is well documented.[40] A huge plethora of scientific and clinical research has led to the approval of the therapeutic virtue of these compounds that exhibit antagonistic activity against oxidative stress, viruses, bacteria, allergies, cancer, cardiovascular diseases, and many other health disorders. For example, anthocyanin that is the most important plant pigment, possessed antitumor, antiulcer, and anti-inflammatory effects.[41]

The application of the design of the experimental method for optimizing extraction revealed a higher sensitivity of the methanol solvent to phenolic compounds. This paralleled previous reports that indicated the *in vitro* radical scavenging properties of methanolic extract and indicated that its antioxidant efficiency contributed to the anti-inflammatory potential.

HPLC profiling of *Opuntia stricta* cladodes' methanolic extract revealed that it contains catechin, 4HO-benz, rutin, verbascoside, luteolin-7-glucose, apigenin-7-glucose, oleuropein, quercetin, pinoresinol, and ellagic acid (Figure 1 and Table 4). Among these compounds, lutelin-7-glucose, apigenin 7-glucose, and catechin were the most represented ones (4.25 μg/g, 3.15 μg/g, and 2.85 μg/g, respectively).

These phenolic compounds are mainly known for their potent antioxidant potential.[1,30,47] They also exhibit variable medical interests, such as anticancer[42−44] and anti-inflammatory activity.[4,45,46] The antioxidative system of living organisms comprises two pathways to eliminate or neutralize harmful free radicals: the enzymatic and chemical ones. Superoxide dismutases (SOD), glutathione peroxidase (GPx), and catalase (CAT) are the most investigated antioxidative metalloproteins

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Table 5. *In Vitro* Antioxidant Activity of Different Extracts of *Opuntia stricta*[a]

	% of inhibition					
extracts	O2.$^-$	OH•	H_2O_2	nitric oxide activity	DPPH	ABTS (mM TE/g DW)
hexane	15.25 ± 2.8[a]	20.45 ± 2.7[a]	57.85 ± 3.2[a]	22.23 ± 3.3[a]	24.64 ± 3.2[a]	0.28 ± 0.13[a]
ethyl acetate	37.34 ± 4.1[c]	46.93 ± 3.4[c]	67.87 ± 4.2[b]	34.51 ± 4.2[b]	37.81 ± 3.8[b]	1.30 ± 0.15[c]
methanol	54.41 ± 5.34[d]	59.18 ± 3.7[d]	81.59 ± 4.7[d]	71.17 ± 5.3[d]	58.38 ± 4.2[c]	1.81 ± 0.06[d]
water	22.31 ± 3.1[b]	35.65 ± 2.9[b]	75.53 ± 5.2[c]	48.53 ± 4.8[c]	53.93 ± 3.9[d]	0.32 ± 0.032[b]

[a]$O2^{\bullet}$: superoxide radical scavenging activity; $OH^{\bullet}$: hydroxyl radical scavenging assay; H_2O_2: hydrogen peroxide scavenging capacity; DW = dry weight; TE: trolox equivalents. Values having different letters on a same column showed significant difference ($p < 0.01$). The results are sorted in increasing order: a < b < c < d. The mass of extract in the reaction mixture was 250 μg.

Table 6. Acute Anti-Inflammatory Effect of the Methanol Extracts of *Opuntia stricta* on Carrageenan-Induced Rat Paw Oedema (n = 6)[a]

	% edema inhibition					% inflammation inhibition				
samples	1 h	2 h	3 h	4 h	5h	1h	2h	3h	4h	5 h
NaCl (0.9%)	38.88 ± 2.8	40.78 ± 1.31	47.2 ± 1.03	41.50 ± 0.5	35.04 ± 1.51	15.48 ± 1.42	20.21 ± 0.75	21.1 ± 1.11	16.83 ± 2.33	15.47 ± 2.3
carr (1%)	80.12 ± 2.39	84.60 ± 7.11	86.51 ± 4.22	72.79 ± 11.1	65.63 ± 2.02	37.24 ± 2.78	40.19 ± 3.1	47.5 ± 2.79	37.27 ± 2.23	35.5 ± 3.60
carr+indo (10 mg/kg)	50.84 ± 6.7	59.53 ± 1.40	69.75 ± 14.65	44.4 ± 16.3	41.67 ± 6.43	26.7 ± 4.22	20.61 ± 2.5	17.46 ± 1.2	14.7 ± 7.7	13.22 ± 2.5
carr+OS (100 mg/kg)	30.10 ±1.2	27.39 ± 2.5	26.24 ± 2.2	20.58 ± 1.4	19.72±1.6	24.2±1.5	25.39 ± 2.3	29.10 ± 3.2	15.72 ± 2.37	12.58 ± 0.5

[a]Data are presented as mean ± SD.

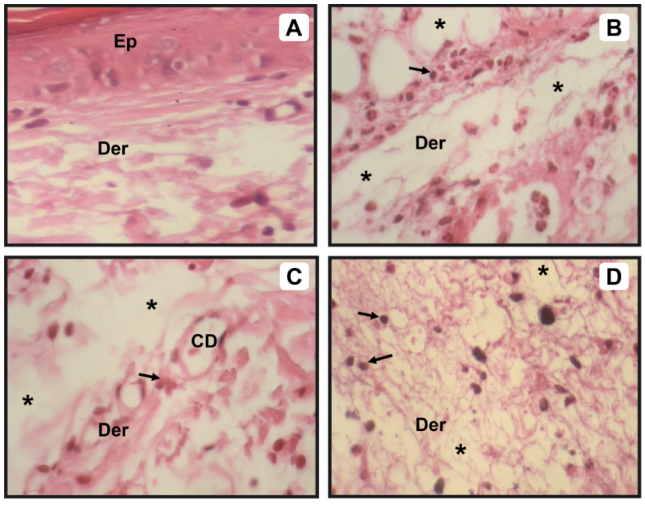

Figure 2. Histological changes in the paw tissue of rats. (A) Paw tissue slice from a sham rat showing the normal tissue structure. (B) Paw tissue slice from a carrageenan-treated rat. (C) Paw tissue slice from a carrageenan-treated rat with indomethacin. (D) The tissue section from a carrageenan-treated rat that received the extract (100 mg/kg). Ep: epidermis, Der: dermis, CD: capillary dilatation, arrows: leukocytes infiltration, asterisks: inflammatory edema. Sections were stained with hematoxylin and eosin (magnification: 400 ×).

Table 7. Binding Affinity of the *Opuntia stricta* Essential Oil Major Identified Compounds with the Different Targeted Receptors: VEGF, IL-6, and TNF-α

	binding affinity (kcal × mol^{-1})		
compound No.	VEGF	IL-6	TNF-α
catechin	−6.3	−8.0	−6.8
tyrosol	−4.2	−5.1	−5.1
4HO-benz	−5.2	−6.1	−6.3
rutin	−6.7	−7.3	−7.5
verbascoside	−6.6	−7.4	−7.6
luteolin-7-glu	−7.1	−7.9	−7.7
apigenin 7-glu	−7.1	−8.7	−7.9
oleuropein	−6.1	−7.5	−6.6
quercetin	−6.7	−7.1	−6.8
pinoresinol	−6.0	−6.9	−6.5
cinnamic	−4.8	−6.1	−5.7
apigenin	−6.3	−7.0	−7.0
ellagic acid	−6.4	−7.1	−7.2

that require metals (selenium, zinc, manganese, copper, and iron) to accomplish their function and can be enhanced by the supply of these elements found in the cladode of *O. stricta*. The second pathway is merely based on chemical reactions of the endogenous produced free radicals with polyphenols and flavonoids (like quercetin and myricetin) that are sought as the principal molecules mediating the antioxidative effects and exist in cactus shrubs.[1,7,44,46]

Antioxidant Potential of the *O. Stricta* Extract. In agreement, our work shows the important antioxidative chemical activity of extracts from nopalitos. The *in vitro* antioxidative assays revealed that all extracts of the cladode have an important scavenging/neutralizing potential against the major oxidizing substances that are distinguished by their proper reactional mechanism.[47] The DPPH scavenging potential was significantly higher ($p < 0.001$) for the methanolic (58.38 ± 4.2%) and aqueous (53.93 ± 3.9%) extracts than the hexane (26.64 ± 3.2%) and ethyl acetate (37.81 ± 3.8%) ones (Table 5). The test showed a significant difference between the different solvents used for extraction. The excessive production of endogenous reactive oxygen substances (ROS), such as superoxide ($O_2^{\bullet -}$), hydroxyl ($OH^{\bullet}$), and hydrogen peroxide (H_2O_2) radicals, leads to oxidative stress that damages cells and organs.[4,7,31,35] Our results revealed the potential of *O. stricta* extracts to inhibit the $O_2^{\bullet -}$ and $OH^{\bullet}$ radicals. The inhibitory effect of the plant extracts ranged from 15.25% to 54.41% for superoxide and from 20.45 to 59.18% for hydroxyl radicals. The highest antioxidant effect was obtained with the methanolic

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Table 8. Number of Conventional H-bonds, Closest Interacting Residues, and Distance to Closest Interacting Residue (Å) of the *Opuntia stricta* L. Identified Compounds, which Possessed the Best Binding Affinities with the Different Targeted Receptors: VEGF, IL-6, and TNF-α

			closest interacting residues		
receptor	compound name	no. conventional H-bonds	interacting residues	closest residue (distance, Å)	no. closest interacting residues
VEGF	luteolin-7-glu	6	**conventional H-bond:** THR^{36}, LEU^{39}, MET^{40}, ARG^{56}, MET^{40}, THR^{36}**carbon H-bond:** LEU^{39}, GLU^{92}**π-cation:** ARG^{56}**π-sulfur:** MET^{93}**π-alkyl:** VAL^{33}	MET40:O (1.999)	7
	apigenin 7-glu	5	**conventional H-bond:** VAL^{37}, VAL^{43}, LEU^{39}, LEU^{39}, THR^{36}**π-sigma:** THR^{36}	THR36:O (1.945)	4
	rutin	8	**conventional H-bond:** GLY^{59}, GLY^{65}, CYS^{68}, GLU^{67}, GLY^{59}, LEU^{66}, GLU^{67}, LEU^{66}**carbon H-bond:** LYS^{106}	GLY59:HN (2.077)	6
IL-6	apigenin 7-glu	4	**conventional H-bond:** CYS^{6}, LEU^{3}, VAL^{93}, ASN^{92}**π-Alkyl:** CYS^{6}, LYS^{29}, LYS^{31}	VAL93:O (2.13)	6
	catechin	4	**conventional H-bond:** GLY^{127}, LEU^{132}, ARG^{154}, ASP^{155}**π−π T-shaped:** PHE^{136}**π-alkyl:** PRO^{157}	LEU132:O (2.092)	6
	luteolin-7-glu	5	**conventional H-bond:** GLY^{126}, THR^{130}, ALA^{152}, THR^{134}, THR^{130}**carbon H-bond:** GLU^{133}**π-anion:** GLU^{129}**π-alkyl:** PRO^{157}	THR13:HN (1.978)	6
TNF-α	apigenin 7-glu	6	**conventional H-bond:** ASN^{46}, GLN^{47}, LYS^{90}, LYS^{90}, LYS^{90}, ALA^{134}**π-anion:** GLU^{135}, GLU^{135}**π-donor H-bond:** ASN^{46}**π-alkyl:** PRO^{139}	GLN47:HE22 (2.269)	6
	luteolin-7-glu	8	**conventional H-bond:** TRP^{28}, TRP^{28}, ASN^{46}, GLN^{47}, ASN^{46}, ALA^{134}, ALA^{134}, ILE^{136}**carbon H-Bond:** PRO^{139}**π-Donor H-Bond:** GLN^{25}**π-Alkyl:** PRO^{139}, PRO^{139}	ILE136:O (2.191)	7
	verbascoside	8	**conventional H-bond:** LEU^{26}, TRP^{28}, ALA^{134}, LEU^{26}, ASN^{46}, ILE^{136}, GLU^{135}, ASN^{92}**carbon H-bond:** ASN^{137}, GLU^{135}**π-anion:** GLU^{135}**π-donor H-bond:** ASN^{46}**π-alkyl:** MTN^{200}	LEU26:HN (1.985)	9

fraction. The potential to scavenge hydrogen peroxide and nitric oxide reaches up to 81 and 71%, respectively, for the methanolic. The total antioxidant capacity was 6-fold more significant with the methanolic fraction than with hexane and water extracts (Table 5). The peculiar antioxidant potential of the methanolic extract was attributed to the high levels of phenols and flavonoids such as catechin, quercetin, apigenin-7-glucose, and luteolin-7-glucose.

Anti-inflammatory and Histopathological Analysis. Because of its richness in polyphenols and flavonoids and its highest antioxidant activity, the methanolic extract of the cladode of *O. stricta* was chosen to test its *in vivo* anti-inflammatory effects. The administration of 100 mg/kg of body weight of the extract to rats with carrageenan- induced inflammation resulted in a significant time-dependent decrease in edema volume to 30.10 ± 1.2% (relatively to the initial status) 60 min following the treatment, to finally reach 19.72 ± 1.6% 4 h later. The inhibitory effect was 2- and 3-fold more important than in the indomethacin-treated (41.67 ± 6.43%) and control (65.63 ± 2.02%) groups (Table 6). The microscopic slides revealed prominent dissociated fibers and infiltration of inflammatory cells in the paw skin of rats injected with carrageenan alone (Figure 2 a). The immune cells' infiltration was reduced by the administration of *O. stricta* extract (100 mg per kg of BW) and indomethacin (10 mg per kg of BW) , but re-establishment of the stromal fiber structure was more noted in the extract-treated rats (Figure 2). The mechanism of carrageenan-induced inflammation involves the release of several proinflammatory, inflammatory, and hyperalgesic factors, such as prostaglandins, kinins, and cathecolamines.[4] The inflammatory-antagonizing effect of *O. stricta* is attributed to its higher content in polyphenols and flavonoids that inhibit the release and functions of inflammatory mediators.[4,48,49] Further contribution in enhancing the wound healing of the injured skin comes from fatty acids through modulating the immune response and fibroblasts regenerative activity.[50,51]

Figure 2 exhibits a paw tissue slice from a carrageenan-treated rat showing oedematasis in the dermis, a spongy-like appearance and a bull illustrates a paw tissue treated with Indomethacin. The tissue section from a carrageenan-inflamed rat, which was also treated by *O. stricta* extract (100 mg/kg PW), demonstrates reduced edematasis without any spongy-like feature and bulla. The extract might significantly reduced myeloperoxidase activity. The effects of cladode powder on inflammation and myeloperoxidase activity were confirmed by histological assays, as the extract decreased the histopathological injury features, including leucocyte infiltration, in rats inflamed by carrageenan injections (acute inflammation). The inflammatory process can be divided into two phases. While the early phase is related to the production of histamine, cyclooxygenase, 5-hydroxytryptamin, and bradykinins, the delayed phase is associated with neutrophil infiltration and the continuous production of arachidonic metabolites.[4,11,51] The *Opuntia stricta* extract was able to attenuate the early and delayed inflammatory phases by a dose range of 100 mg/kg. Cladodes of *O. ficus-indica* extracts lowered cholesterol levels, conveyed antiulcer potentials, and their aqueous extract significantly improved the wound healing process[52] and detoxified the body.[7]

***In Silico* Modeling Results.** The *in silico* modeling approach revealed that *O. stricta* compounds had various affinities for each of the assessed receptors: VEGF, IL-6, and TNF-α (Table 7). Several reports indicated that variation in binding affinities is mainly the result of the chemical composition and geometry of the studied ligands.[30,31,43] In this study, all *O. stricta* compounds had negative binding scores, which supported their potential biological activities. These binding affinities reached −7.1 kcal/mol for VEGF, −8.7 kcal/mol for IL-6, and −7.9 kcal/mol for TNF-α. The best binding affinity was predicted for apigenin-7-Glu (−8.7 kcal/mol) and then for catechin (−8.0 kcal/mol), while complexed with IL-6 macromolecules, which may contribute to their highest biological activities, specifically the anti-inflammatory and healing effects (Table 8 and Figure 3). Apeginin 7-Glu possessed the best binding score for the studied receptors: VEGF, IL-6, and TNF-α. *O. stricta* compounds established good molecular interactions with the targeted receptors that showed up-to 8 conventional H-bonds supported with a network of carbon H-bonds, hydrophobic, and/or electrostatic bonds that contributed to the stability of the

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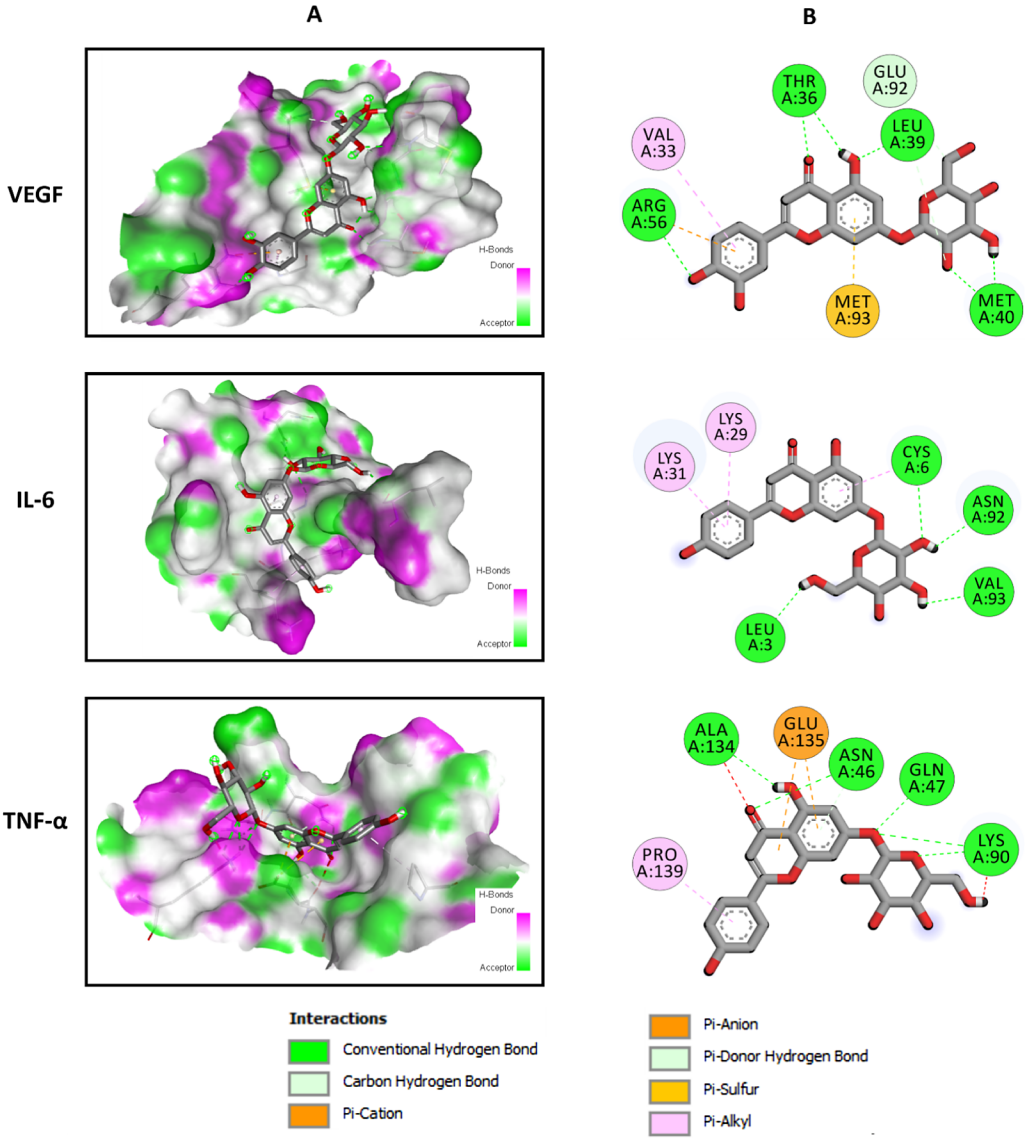

Figure 3. Illustrations of 3D docked ligands (A) and their corresponding 2D diagram of interactions (B) of *Opuntia stricta* L. compounds, which possessed the best binding affinities with the different targeted receptors: Luteolin-7-Glu complexed with VEGF and Apigenin 7-Glu complexed with both IL-6 and TNF-α.

complexes.[4,7,30,31] These molecular interactions included several key residues.

The identified *O. stricta* compounds were also deeply embedded in the targeted receptors. Here again, apigenin 7-Glu showed the deepest embedding while docked to VEGF. In fact, the distance was 1.945 Å only from the THR[36] residue. It could be deduced that the calculated binding energies, the molecular interactions, and the deep embedding of *O. stricta* compounds confirm that the potential anti-inflammatory and healing process effects are thermodynamically possible. These effects have already been approved by both *in vitro* and *in vivo* approaches on antioxidant and anti-inflammatory effects in *Wistar* rats. Our results also confirm the promising nutraceutical and health benefial effects of the medicinal plants and the natural-derived compounds, including *Opuntia* plants.[7,11,30,31,43,53] These effects may also include the acceptable bioavailability and pharmacokinetic properties of the *O. stricta* identified phytochemicals.

CONCLUSION

O. stricta is highly enriched in nutrients, such as copper, zinc, manganese, iron, and different types of fatty acids, that contribute not only to the metabolic process but also enhance the protection of cells against oxidative stress and inflammation. It also contains important amounts of phenolic compounds, particularly in the methanolic extract that are endowed with great scavenging potential against free radicals. The binding affinities and molecular interactions of *O. stricta* compounds may support the experimental *in vitro* and *in vivo* findings, which may result in antioxidant and antiinflammatory activities. Our findings endorse the usage of the plant for antioxidant purposes and anti-inflammation.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.4c04330.

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(A) Standardized Pareto chart for efficiency; (B) factor levels for a 2^4 factorial design; (C) effects of the variation of the four main parameters on polyphenolic content efficiency; (D) response surface for the polyphenolic content as a function of the solvent using and maceration period (PDF)

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Notes

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