CHEMISTRY & BIODIVERSITY

Accepted Article

Title: Analyzing the Bioactive Properties and Volatile Profiles Characteristics of Opuntia dillenii: Exploring its Potential for Pharmacological Applications

Authors: Loukili El Hassania, Kadi Mounime, Amine Elbouzidi, Mohamed Taibi, Chebaibi Mohamed, Legssyer Abdelkhaleq, Ramdani Mohamed, Hanae Naceiri Mrabti, Gokhan Zengin, Mohamed Addi, and Marie-Laure Fauconnier

To be cited as: *Chem. Biodiversity* **2024**, e202301890

Link to VoR: https://doi.org/10.1002/cbdv.202301890

www.cb.wiley.com

WILEY-VHCA

10.1002/cbdv.202301890

Analyzing the Bioactive Properties and Volatile Profiles Characteristics of *Opuntia dillenii (Ker Gawl.)* Haw: Exploring its Potential for Pharmacological Applications

Loukili El Hassania [1,2]*, Kadi Mounime [3], Amine Elbouzidi [1,4], Mohamed Taibi [4,5], Chebaibi Mohamed [6], Legssyer Abdelkhaleq [3], Ramdani Mohamed [1], Hanae Naceiri Mrabti [7], Gokhan Zengin[8*], Mohamed Addi [4], and Marie Laure Fauconnier [9]

[1] Euromed Research Center, Euromed Polytechnic School, Euromed University of Fes, Route de Meknès (Rond Point Bensouda), 30 000 Fès, Morocco

[2] Laboratory of Applied Chemistry & Environment, Faculty of Sciences, University Mohammed 1st, Bd. Med VI B.P. 717, Oujda, Morocco.

[3] Laboratory of Bioresources, Biotechnology, Ethnopharmacology and Health, Faculty of Sciences, University Mohammed 1st, Bd. Med VI B.P. 717, Oujda, Morocco;

[4] Laboratoire d'Amélioration des Productions Agricoles, Biotechnologie et Environnement (LAPABE), Faculté des Sciences, Université Mohammed Premier, Oujda 60000, Morocco.

[5] Centre de l'Oriental des Sciences et Technologies de l'Eau et de l'Environnement (COSTEE), Université Mohammed Premier, Oujda 60000, Morocco

[6] Ministry of Health and Social Protection, Higher Institute of Nursing Professions and Health Techniques, Fez, Morocco;

[7] High Institute of Nursing Professions and Health Techniques Casablanca, Morocco.

[8] Department of Biology, Science Faculty, Selcuk University, 42130 Konya, Turkey

[9] Laboratory of Chemistry of Natural Molecules, University of Liège, Gembloux Agro-Bio Tech. 2, Passage des Déportés, B-5030 Gembloux, Belgium;

*Corresponding authors: Dr. Loukili El Hassania – e.loukili@ump.ac.ma; Prof. Dr. Gokhan Zengin – gokhanzengin@selcuk.edu.tr; Prof. Dr. Mohamed Addi – m.addi@ump.ac.ma

Academic titles of each author:

Dr. Loukili El Hassania – e.loukili@ump.ac.ma
Dr. Kadi Mounime - kmounime@ump.ac.ma
Dr. Amine Elbouzidi- amine.elbouzidi@ump.ac.ma
Dr. Mohamed Taibi - mohamedtaibi9@hotmail.fr
Dr. Chebaibi Mohamed - mohamedchebaibi@yahoo.fr
Prof. Dr. Legssyer Abdelkhaleq - a.legssyer@yahoo.fr
Prof. Dr. Ramdani Mohamed - moha.ramdani2000@yahoo.fr
Prof. Dr. Hanae Naceiri Mrabti - naceiri.mrabti.hanae@gmail.com
Prof. Dr. Gokhan Zengin – gokhanzengin@selcuk.edu.tr
Prof. Dr. Mohamed Addi – m.addi@ump.ac.ma
Prof. Dr. Marie Laure Fauconnier - marie-laurefauconnier@uliege.be

Highlights:

- The research centered on investigating the chemical composition, the antioxidant, and the anti-inflammatory activity of oils and pulpy variants (Imatchan, Harmocha, and Aknari) sourced from *O. dillenii.* The investigation encompassed both *in vitro* and *in silico* analyses.
- Aldehydes were predominant in *Opuntia* pulp, accounting for 57.33% to 68.25%, and in *Opuntia* oil, ranging from 48.92% to 64.7%. E-11-hexadecenal and (E)-2-undecenal were found in significant quantities in *Opuntia* oil and fruit.
- The oils demonstrated anti-inflammatory activity based on BSA, LOX, Hypotonia, and Heat assays. With AK variant oil having a LOX inhibition with an IC_{50} of 56.72 ± 3.97 µg/mL, followed by HA oil, and IM oil. AK oil showed concentration-dependent inhibition of RBC membrane hemolysis, reaching 74.31% at 250 mg/mL. AKO, HAO, and IMO oils demonstrated inhibition of heat-induced hemolysis. IM oil demonstrated the most potent inhibitory effect on albumin denaturation.
- The most noteworthy antioxidant results include the significantly lower DPPH IC_{50} values for *O. dillenii* oils in the IM variant (15.17 ± 1.28 mg/mL), reduced FRAP IC_{50} values for the AK variant (30.23 ± 0.6 mg/mL), and the remarkable ferric ion chelating activity of the HA variant with an IC_{50} value of 39.54 ± 0.59 mg/mL.

10.1002/cbdv.202301890

Abstract:

In this investigation, the study focused on the chemical constitution and the antioxidative as well as anti-inflammatory characteristics of oils and pulpy variants (Imatchan (IM), Harmocha (HA), and Aknari (AK)) sourced from *O. dillenii*. This inquiry encompassed both *in vitro* and *in silico* analyses. High-performance liquid chromatography (HPLC) was employed to ascertain the phenolic constituents, while gas chromatography-mass spectrometry (GC-MS) methodologies. were applied to discern the volatile makeup. The appraisal of antioxidant potential was conducted via the deployment of assays such as 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP), and ferric ion chelating (FIC) techniques. The anti-inflammatory activity was examined using BSA and LOX. Molecular docking methods assessed the antioxidant and anti-inflammatory properties. According to HPLC findings, the most abundant compounds detected in AKO and IMO cultivars were quercetin 3-*O*-β-D-glucoside followed by vanillic acid, ferulic acid and tyrolsol. Concerning headspace GC-MS analysis E-11-hexadecenal and (E)-2-undecenal contribute to the major compounds detected in *Opuntia* HA, IM, and AK pulp and oil. The DPPH IC_{50} for AK, HA and IM were 38.41±1.54, 42.24±0.29 and 15.17±1.28 mg/mL, respectively. The FRAP IC_{50} capacity of AK, HA and IM was determined to be 30.23 ± 0.6, 55.96 ± 0.08 and 23.41 ± 1.83 mg/mL, respectively. AK, HA and IM displayed significant FIC activity, with IC_{50} values of 42.75 ± 0.63, 39.54 ± 0.59 and 35.31 ± 1.38 mg/mL, respectively. The AK, HA and IM *O. dillenii* oils were effective in their anti-inflammatory activity. Molecular docking of *O. dillenii* oils phenolic compounds was conducted to determine the possible targeted proteins by the phenolic compounds in *O. dillenii*'s compounds. Overall, these fruits demonstrated the potential for new ingredients for culinary or pharmaceutical applications, providing value to these natural species that can flourish in arid conditions.

Keywords: *Opuntia dillenii*, oil, phenolic compounds, volatile compounds, antioxidant and Anti-inflammatory activity

Introduction

The discoveries in the field of medicinal plants and polyphenols continue to evolve, paving the way for exciting new research [1]. Scientists are actively exploring the diversity of plants to identify new beneficial compounds and are delving deeply into the mechanisms by which these compounds affect our health. This quest for knowledge offers a promising perspective for the development of future medical treatments [1]. The traditional use of medicinal plants in many cultures around the world also highlights the historical importance of these natural resources in medicine. Ancestral practices often serve as the starting point for modern research on medicinal plants, underscoring the importance of preserving this traditional knowledge [2]. In the end, medicinal plants and polyphenols represent a valuable source of natural substances that can contribute to our overall well-being. There are numerous functions of polyphenols, such as antioxidant, antiviral, anti-inflammatory, and anticancer activities [3]. These activities are primarily attributed to these compounds' ability to reduce free radicals and their affinity for various proteins, including enzymes and receptors. Polyphenols support natural defenses by protecting tissue components (lipids and other macromolecules) from oxidative damage and cellular aging as antioxidants. Their ingestion results in a momentary increase in plasma antioxidant capacity in the hours following a meal. According to numerous studies, polyphenols may contribute to reducing chronic diseases related to osteoporosis, cardiovascular and degenerative disorders, and cancer [4].

The characterization of phenolic compounds is particularly interesting due to their antioxidant [5], neuroprotective, anti-inflammatory, cardioprotective, hepatoprotective, and antidiabetic properties [6, 7]. However, *Opuntia* contains significant quantities of volatile compounds [8], which are also of interest due to their increasing application in the food and pharmaceutical industries.

Volatile organic compounds (VOCs) exhibit diverse functionalities within biological systems. For instance, as delineated by Schwab et al. [9], VOCs exert an influence on fruit fragrance amplification while concurrently bestowing protection against herbivores, microbial proliferation, and abiotic stressors. Furthermore, VOCs emanating from fruits serve as a magnet for organisms involved in the dispersal of seeds. Within the context of flavor and aroma augmentation, volatile organic constituents manifest in both unconstrained and tethered configurations. In the latter scenario, VOCs, through β-glycosidic linkages to mono- or disaccharides, are present as latent precursors, underscoring their non-volatile nature. Conversely, unbound VOCs contribute directly to the olfactory attributes of fruits [10].

The developmental phases of fruit growth and maturation exert a notable influence on volatile organic compounds (VOCs), inducing both qualitative and quantitative modifications intricately linked to the liberation of VOCs often stored as glycosylated entities [11]. Consequently, the aromatic fingerprint can serve as a discerning identifier to discriminate distinct stages of fruit ripening as well as diverse genetic origins of the fruits [12].

To maximize the nutritional value of our various *O. dillenii* oils, the study conducted a comprehensive phytochemical evaluation (GC-MS and HPLC in the headspace) as well as several biological tests, including antioxidant and anti-inflammatory activity. Their *in silico* anti-inflammatory and antioxidant potential was assessed. The Pro-Tox II web server was used to assess their potential toxicity. This will allow us to determine whether we are dealing with a synergy of active molecules or a single active principle responsible for a specific biological activity. The results obtained should contribute to promoting the use of these fruits as sources of functional ingredients for the food sector or other related applications.

Results and discussions

Volatile compounds

Volatile compounds are chemicals with a low boiling point and evaporate easily at room temperature. They are often used in the fragrance and flavor industries, as well as in some industrial applications. To determine the composition and concentration of VOCs in samples, Headspace, gas chromatography (GC) and mass spectrometry (MS) are used to extract volatile compounds from a sample, trapping them in an airtight container and analyzing the gases above the sample. This method is used to identify and quantify the volatile compounds in the samples, which can provide information on the plant extract's quality, freshness, and authenticity. Some volatile compounds in plant extracts include terpenes, alcohols, aldehydes, and esters, which can contribute to the extract's flavor, aroma, and medicinal properties. So for our investigation, Table 1 and Figure 1 display the analysis results of the volatile aroma compounds present in Three different *O. dillenii* samples. A total of 65 volatile compounds were detected and classified into six main categories based on their functional groups: esters (10 compounds), alcohols (26 compounds), aldehydes (18 compounds), hydrocarbons (2 compounds),

ketones (7 compounds), terpenes (1 compound) and ether (1 compound). The findings showed significant variations in the quantity and number of volatile compounds in Three different *O. dillenii* samples. Aldehydes have been previously identified as a contributor to the aroma of *O. dillenii*. while aldehydes and alcohols were found in high amounts in *Opuntia* Oil and fruit (HA, AK and IM). Aldehydes were the most prevalent type of volatile organic compounds (VOCs), as shown by their respective proportions of 57.33 ± 0.24%, 68.25 ± 0.7%, and 55.50 ± 0.11% of total VOCs in HA, IM, and AK *Opuntia* pulp, and 48.92 ± 0.7%, 64.7 ± 0.11% and 62.52 ± 0.2% of total VOCs in HA, IM, and AK *Opuntia* oil. The aldehyde content in both IM and AK *Opuntia* pulp significantly increased. At the same time, there was a significant decrease in aldehyde levels in HA *Opuntia* oil compared to the other samples (as shown in Figure 1).

Numerous aldehydes were identified in *Opuntia* samples, among which E-11-hexadecenal and (E)- 2-undecenal contribute to the major compounds detected in *Opuntia* HA, IM, and AK pulp and oil (Table 1). Other aldehydes present in the cultivar *Opuntia* were detected at an exceptional level, but the pulp was the most dominant of these compounds compared to the oil.

For another profile, following aldehydes, alcohols are present in large amounts in our samples. The alcohol concentration in IM, AK, and HA is considerable at 30.74 ± 0.14%, 34.12 ± 0.6% and 16.74 ± 0.74%, respectively, for AK, IM and HA. The HAO cultivar showed higher volatile chemical contents (30.36 ± 0,6%) than the IM, AK and HA Oil varieties. (E)-2-Tridecen-1-ol, was previously reported to be one of the major contributors to the *Opuntia* aroma. In comparison, the concentration of the latter was negligible in HAO variety. The alcohols are most commonly found in fruits, and their levels increase as the variety and its concentration decrease in oils compared to the pulp variety. This could be attributed to the conversion of aldehydes to alcohols during fruit ripening with the help of alcohol dehydrogenase and alcohol acyl-CoA reductase enzymes [13].

Among several forms of esters identified in the *Opuntia* variety (Table 1), isopropyl hexadecanoate was previously reported to be one of the most ester contributors to the *Opuntia* aroma (AKF, AKO and HAF) and its concentration (3.38, 3.16 and 3.55 %, respectively) decreased with others variety. In the HA variety, 1-hexanol and (E)-2-hexen-1-ol were the most abundant ester identified in HA oil (13.08 and 6.64 and their concentration showed a decreasing tendency as the variety of *Opuntia* (pulp and oil) changed.

In the present study, Ketones were found in small amounts in the different fruit varieties. However, the ketones, including 2-isopropyl-5-methyl cyclohexanone and Z, Z-6,28-heptatriactontadien-2-one, were the most abundant volatile aroma compounds found in *Opuntia* varieties. Two hydrocarbon molecules were also discovered in trace amounts. Among these, 1-ethyl-3,5-dimethyl-benzene and 1,2-diisopropenylcyclobutane were detected only in AK fruit with levels of 2.81 and 4.57, respectively.

According to Chahdoura et al., findings, camphor (49%), nonanal (16.4%) ethyl acetate (54%), 1-hexyl acetate (15.3%) and 1-nonanol (22%) were the major volatile compound detected in skins and pulps of *O. microdasys* and *O. macrorhiza* using HS-SPME extraction method [14]. Zito et al. Hydrocarbons (43.5%), aldehydes and ketones (21.8%), and hydrocarbons (21.4%) made up the majority of the chemical groups in oils from the fruit's red skin. In contrast, the oils from the yellow skin and cactus pear (*Opuntia ficus indica* L.) flowers have a low level of these compounds. The main volatile ingredients are benzenacetate aldehyde, D-3-carene, hexanol, and α-pinene [15]. According to González-Aguero et al.. as the fruit grows, tissue damage makes membrane lipids more accessible to lipoxygenase enzymes. Which then causes them to produce saturated and unsaturated volatile C6 aldehydes and alcohols.

10.1002/cbdv.202301890

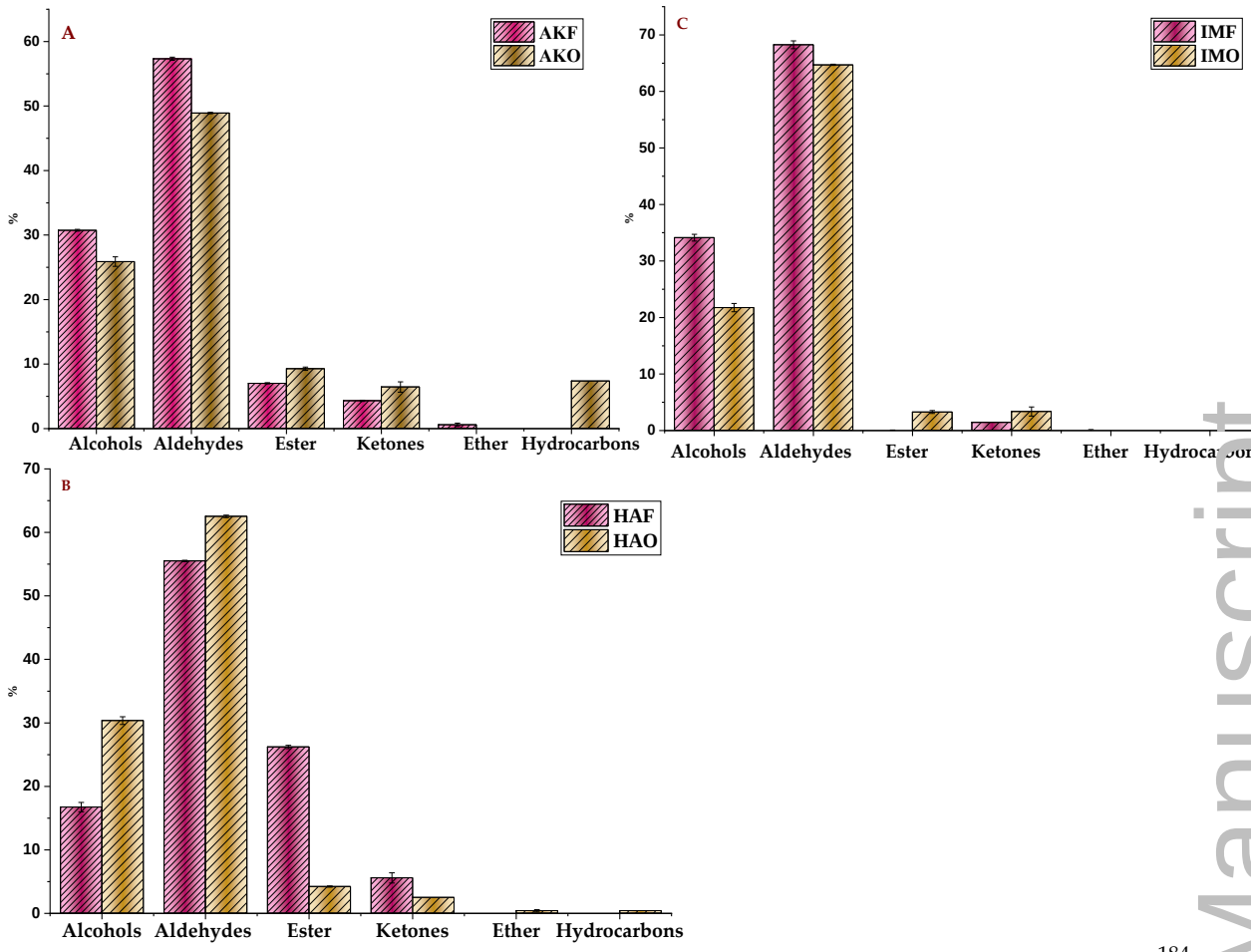


Figure 1. Content of volatile compounds classes contained in oil and pulp *O. dillenii*.

10.1002/cbdv.202301890

Table 1. Volatile organic compounds (VOCs) detected using HS/SPME and GC/MS obtained from *O. dillenii* fruit

N°	Volatile Compounds	RT (min)	AK (%)		HA (%)		IM (%)		RI
	Alcohols		**Oil**	**pulp**	**Oil**	**pulp**	**Oil**	**pulp**	
1	1,6-octadien-3-ol	6.13±0.10	1.04 ±0.01	nd	4.03± 0.01	nd		nd	876
2	1-hexadecanol	13.57±0.52	nd	0.46±0.01	0.2±0.01	nd	nd	nd	1428
3	2-dodecyl-1,3-propanediol	9.97±0.00	1.01±0.00	0.37 ±0.00	0.46±0.00	1.68±0.00	nd	nd	1755
4	2-ethylnon-1-en-3-ol	12.13± 0.00	0.89±0.06	1.19±0.06	0.82±0.06	1.23±0.06	nd	nd	1419
5	Z-11,13-tetradecadien-1-ol	5.47 ± 0.00	nd	nd	nd	7.89±0.00	2.67±0.00	nd	1633
6	2-hexyl-1-octanol	12.43± 0.00	nd	0.32±0.00	nd	nd	nd	nd	746
7	2-octadecyl-propane-1,3-diol	15.29± 0.00	0.38±0.00	0.63±0.00	0.33±0.00	nd	nd	nd	1659
8	4-ethylcyclohexanol	9.83± 0.09	0.52±0.11	0.32±0.11	0.4±0.11	nd	nd	nd	1883
9	2,2,4-trimethyl-5-hexen-3-ol	6.83 ± 0.06	nd	0.67±0.01	1.01±0.01	nd	nd	nd	1403
10	5-isopropyl-2-methylbicyclo[3,1,0] hexan-2-ol	5.23 ± 0.14	0.26±0.08	nd	nd	nd	nd	nd	998
11	6,6-dimethyl-cyclohex-2-en-1-ol	15.74± 0.00	0.52±0.02	1.02±0.02	0.8±0.02	nd	nd	0.39±0.00	695
12	6-methyl-bicyclo[4,2,0]octan-7-ol	12.85± 0.00	1.31±0.00	0.39±0.00	0.18±0.00	nd	nd	nd	1915
13	2,3,6-trimethyl-7-octen-3-ol	8.47 ± 0.06	0.38±0.00	0.53±0.00	0.73±0.00	nd	nd	nd	1695
14	9-octadecen-1-ol	13.99 ±0.00	0.4±0.00	0.52±0.00	0.35±0.00	nd	3.37±0.00	nd	1239
15	9-oxa-bicyclo[3,3,1]nonane-1,4-diol	9.45 ± 0.01	3.29±0.00	2.88±0.00	2.58±0.00	nd	2.38±0.00	nd	1788
16	2,6,6-trimethyl-bicyclo [3,1,1]heptan-3-ol	15.20± 0.20	nd	0.33±0.00	0.14±0.00	nd	nd	0.41±0.00	1360
17	cycloheptadecanol	16.52± 0.24	0.32±0.00	0.46±0.00	nd	nd	nd	nd	1877
18	4-(1-methylethyl)-cyclohexanol	11.05± 0.11	nd	0.93±0.00	0.88±0.00	nd	1.31±0.00	nd	1967
19	Z,Z,Z-1,3,12-nonadecatriene-5,14-diol	16.31± 0.00	0.59±0.00	0.85±0.00	0.47±0.00	nd	nd	nd	1531
20	E-2-tetradecen-1-ol	15.97± 0.02	0.58±0.01	nd	nd	nd	nd	nd	1953
21	heptanol	5.95 ± 0.00	1.93±0.00	1.98±0.00	3.19±0.00	0.69±0.00	2.19±0.00	3±0.00	1930
22	trans-9-hexadecen-1-ol	13.22± 0.00	0.57±0.00	0.68±0.00	0.24±0.00	nd	0.78±0.00	1.1±0.00	842
23	(E)-2-tridecen-1-ol	8.07± 0.00	11.46±0.0	14.91±0.00	17.02±0.0	nd	17.20±0.00	16.25±0.0	1726
24	Z-2-octadecen-1-ol	12.63± 0.00	0.45±0.09	0.84±0.09	0.33±0.09	nd	1.66±0.09	0.6±0.04	1185
25	2-pentyl-cyclohexane-1,4-diol	15.06± 0.04	nd	0.46±0.04	0.23±0.04	nd	nd	nd	1677
	Aldehydes								
26	heptanal	4.59 ± 0.06	3.87±0.00	5.21 ± 0.00	8.57±0.00	5.07±0.00	7.17±0.00	6.93±0.00	659
27	(8Z)-14-methyl-8-hexadecenal	10.56± 0.00	8.51±0.00	nd	nd	nd	14.33± 0.0	nd	1482
28	(E)-2-octen-1-al	7.31 ± 0.02	4.86±0.00	1.94 ± 0.00	2.93±0.00	nd	2.36±0.00	2.5±0.02	1075
29	13-tetradecenal	17.46± 0.00	0.49±0.02	0.76 ± 0.02	0.42±0.02	nd	nd	nd	2026
30	1-octanal	7.55± 0.01	2.58±0.00	3 ± 0.00	4.43±0.00	2.87±0.00	8.48±0.00	3.06±0.00	1111

10.1002/cbdv.202301890

31	(E,E)-2,4-dodecadienal	11.17± .01	2.32±0.00	0.71 ± 0.00	0.37±0.00	6.4±0.00	2.56±0.00	1.13±0.24	1543
32	Z-2-decenal	10.31±0.01	8.71±0.24	1.54 ± 0.24	0.85±0.24	11.94±0.24	nd	1.35±0.00	1455
33	E-2-nonenal	8.96 ± 0.26	2.51±0.00	2.92 ± 0.00	3.47±0.00	nd	3.24±0.00	3.37±0.14	1301
34	2-undecenal	11.81± 0.02	0.95±0.14	1.4 ± 0.14	1.2±0.14	12.68±0.14	14.39±0.14	14.52±0.1	1604
35	7-hexadecenal	19.00± 0.19	nd	nd	nd	12.05±0.16	0.16±0.00	nd	2117
36	(E)-2-undecenal	10.60± 0.01	nd	12.27± 0.17	11.95±0.1	nd	14.39±0.17	16.98±0.1	1486
37	E-11-hexadecenal	11.92± 0.00	8.33±0.19	13.76± 0.19	11.64±0.1	0.97±0.19	nd	nd	1614
39	E-14-hexadecenal	18.35± 0.14	nd	nd	nd	1.67±0.00	nd	nd	2080
40	E-15-heptadecenal	16.03± 0.00	0.4±0.02	nd	nd	nd	nd	nd	1934
41	hept-2-enal	5.74 ± 0.05	1.8±0.06	2.1 ± 0.06	2.55±0.06	nd	0.49±0.06	3.08±0.14	801
42	hexadecanal	9.65 ± 0.00	1.13±0.14	1.55 ± 0.14	1.47±0.14	nd	0.4±0.14	1.36±0.00	1383
43	octanal	6.412 ± 0.01	2.46±0.00	8.21 ± 0.00	10.75±0.00	nd	0.44±0.00	8.16±0.05	927
44	undecanal	11.40± 0.08	nd	1.96 ± 0.05	1.92±0.05	1.85±0.05	nd	2.26±0.00	1565
	Ester								
45	(2Z)-2-octadecenyl acetate	13.44± 0.01	0.60± 0.26	0.85 ± 0.26	0.18±0.26	nd	nd	nd	1744
46	(4Z,16Z)-4,16-octadecadienyl acetate	18.57± 0.17	0.68± 0.04	1.36 ± 0.04	0.49±0.04	nd	nd	nd	2092
47	3,7-dimethyl-, acetate	6.13 ± 0.26	1.04± 0.01	nd	nd	4.03±0.01	2.04 ±0.01	nd	876
48	1-ethyldecyl acetate	7.95 ± 0.00	nd	1.65 ± 0.00	nd	nd	nd	nd	1168
49	2-propyltetrahydro-2H-pyran-3-yl acetate	7.86 ± 0.25	1.72 ± 0.06	nd	nd	13.08±0.06	nd	nd	1156
50	benzyl 2-hydroxybenzoate	10.84± 0.00	1.04 ± 0.01	nd	6.64 ± 0.09	nd	1.75±0.02	nd	1510
51	methyl 5-oxotridecanoate	4.40 ± 0.02	nd	nd	nd	0.85±0.02	nd	nd	693
52	nonanoic acid, 1-methylethyl ester	10.84± 0.06	1.87± 0.01	nd	nd	0.65±0.01	nd	1.53±0.01	1510
53	octanoic acid, tetradecyl ester	10.10± 0.00	nd	nd	nd	0.95±0.01	nd	nd	1433
54	3,7-dimethyloct-6-enyl Acetate	19.34 ±0.00	3.38± 0.25	3.16 ± 0.25	3.55± 0.25	nd	nd	nd	2136
	Ketones								
55	2-methyl-cyclododecanone	14.56± 0.09	0.72± 0.00	1.01 ± 0.00	0.53± 0.00	nd	nd	0.85±0.01	1831
56	2-isopropyl-5-methylcyclohexanone	9.10 ± 0.04	5.39± 0.01	nd	nd	nd	1.43 ± 0.01	nd	1318
57	6-methylhept-5- en-2-one	12.50± 0.00	nd	nd	nd	0.83 ± 0.01		nd	1665
58	gamma dodecalactone	10.64± 0.00	nd	1.24	1.13± 0.01	nd	0.78 ± 0.01	0.24±0.20	1490
59	Z,Z-6,28-heptatriactontadien-2-one	18.37± 0.16	0.34	1.28	0.59± 0.00	4.76 ± 0.00	nd	nd	2081
	Ether								
60	ether, hexadecyl vinyl	14.27± 0.00	nd	0.6	0.41± 0.02	nd	nd	nd	1809
	Others								

10.1002/cbdv.202301890

61	1-ethyl-3,5-dimethyl- benzene	6.75 ± 0.00	2.81± 0.34	nd	nd	nd	nd	nd	985
62	1,2-diisopropenylcyclobutane	6.94 ± 0.09	4.57± 0.21	nd	nd	nd	nd	nd	1016
63	2-methyl-7-oxabicyclo [2,2,1] heptane	12.29± 0.00	0.64± 0.05	nd	nd	nd	nd	nd	1647

nd: not detected; **Tr:** retention time; **AK:** Aknari, **IM:** Imtchan **HA:** Harmocha

10.1002/cbdv.202301890

The analysis of phenol compounds of Moroccan *Opuntia* fruits using the HPLC technique resulted in the identification of 14 compounds (Table 2). The analysis of components in oil extracts and their separation based on their physical and chemical properties. This method can also be used to determine the presence of specific compounds in a sample, as well as to quantify their concentrations (Figure 2).

Table 2. Phenolic compounds obtained from *O. dillenii* oil

N°	Compounds (mg/100 g)	Tr	AKO	HAO	IMO
1	**Catechin**	7.32	7.65	6.71	1.05
2	**4-Hydroxybenzoic acid**	7.58	5.08	1.47	**10.52**
3	**Tyrolsol**	8.44	7.83	**22.23**	7.38
4	**Caffeic acid**	8.73	2.82	3.51	1.26
5	**Vanillic acid**	9.26	1.70	3.35	**25.59**
6	**Vanillin**	9.42	2.33	3.60	5.08
7	***p*-Coumaric acid**	9.78	3.43	nd	nd
8	**Sinapic acid**	10.11	9.69	3.72	nd
9	**Ferulic acid**	10.44	**24.91**	7.31	nd
10	**Quercetin 3-*O*-β-D-glucoside**	11.94	2.95	**25.74**	**38.92**
11	**Rutin**	11.40	**15.62**	2.49	8.77
12	**Quercetin**	12.33	3.43	7.16	nd
13	**Cinnamic acid**	12.85	nd	3.72	nd
14	**Kaempferol**	13.50	12.57	4.31	3.42

nd : not detected

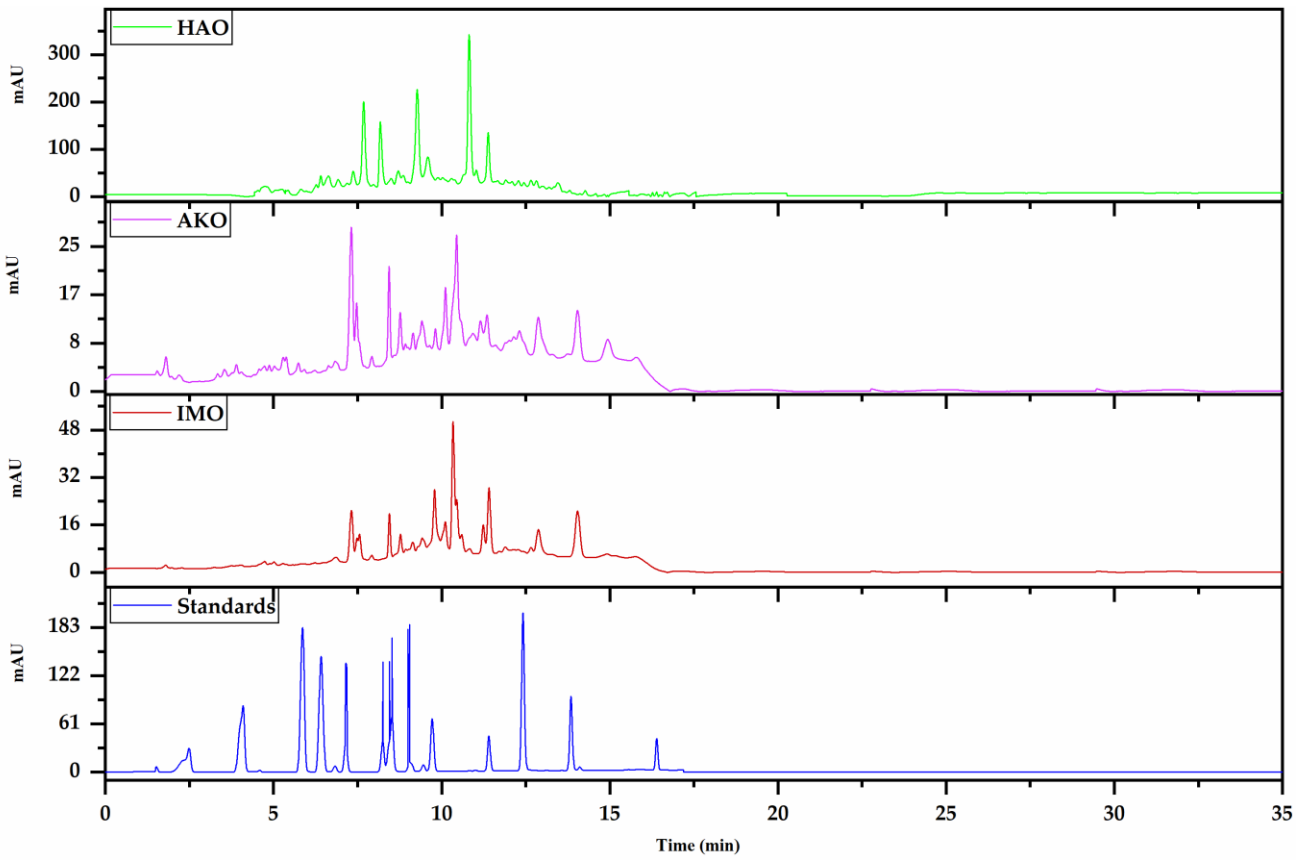


Figure 3. Representative chromatogram of phenolic compounds obtained from *O. dillenii* fruit

10.1002/cbdv.202301890

The comparison between the chemical compositions of the phenolic compounds of the three cultivars, and HAO differed qualitatively and quantitatively concerning AKO and IMO cultivars. Table 2 shows the total phenolic content of aqueous extracts derived from *O. dillenii* gathered in Morocco in mg/per 100g dry matter. The most abundant compound detected in AKO and IMO cultivars was quercetin 3-*O*-β-D-glucoside, with a content of 25.74 and 38.92 mg/100g, respectively. While it was a low quantity in the AKO variety (2.95 mg/100g), followed by vanillic acid (25.59 mg/100g) detected in IMO and it exists in limited quantity in AKO and HAO variety with a level of 1.70 and 3.35 mg/100g, respectively. Ferulic acid and tyrolsol were identified as AKO and HAO's third and fourth compounds. They have a similar amount of phenols with 24.91 and 22.23 mg/100g, respectively. Rutin was a modest compound identified in AKO (15.62± 0.03 mg/100g). In addition, Kaempferol was detected in the AKO variety with a content of 12.57 mg/kg. It is found in the other varieties, HAO and IMO, but only in trace amounts. Other compounds with low values have been identified. Catechin, quercetin and vanillin were present in amounts of 7.65±0.06, 7.16± 0.07 and 5.08±0.05 in AKO, HAO and IMO, respectively. These oils' contents were in agreement with those found in the literature. Ghanya Al-Naqeb et al., found that vanillin, 4-hydroxybenzaldehyde, vanillic acid, and hydroxytyrosol were the most phenols compounds detected in OFI and OD oils [16]. A previous study used HPLC to identify the phytochemicals in oil extracted from OD seeds. For example, loukili et al. discovered that methanolic OD seeds extract contains significant amounts of syringic acid, vanillic acid, kaempferol and quercetin 3-O-β-D-glucoside [17]. According to Jelena et al., quercetin and kaempferol were only in trace amounts in seed and juice extracts. Myricetin was also found in trace amounts in all extracts [18]. The analyzed seed oil identified Tyrosol, vanillin, ferulic acid, pinoresinol, and cinnamic acid as major phenolic compounds [19]. In another study evaluated by Nadia Chougui et al., eight major peaks were selected to identify which Feruloyl-sucrose isomer three and Feruloyl derivative are the main compounds detected in seed oil [20].

10.1002/cbdv.202301890

Anti-inflammatory drugs, both steroidal (glucocorticoids) and non-steroidal (diclofenac), are currently used to treat inflammation. Although effective, these molecules often have adverse effects, which may hinder their long-term use [21], whereas phytochemicals are useful and without side effects [22]. *Opuntia dillenii* has pharmacological power with numerous therapeutic indications. The anti-inflammatory activity of three *O. dillenii* oil seed varieties was evaluated via the lipoxygenase (LOX) test and the bovine serum albumin (BSA) anti-denaturation method Table 3.

Table 3. The anti-inflammatory activity (LOX: lipoxygenase, BSA: bovine serum albumin) of three *O. dillenii oil* seed varieties

Organs and Standards	Anti-inflammatory Activity IC_{50} (µg/mL)	
	BSA	LOX
AK	43.17 ± 0.17	56.72 ± 3.97
HA	62.0 ± 10.19	74.59 ± 4.23
IM	33.34 ± 0.18	91.56 ± 5.47
Diclofenac	83.94 ± 1.53	-
Quercetin	-	71.41 ± 2.11

LOX: lipoxygenase, **BSA:** Bovine Serum Albumin

Lipoxygenase inhibitory activity

Lipoxygenase inhibitory activity was used to examine the anti-inflammatory potential of *O. dillenii* oil seed. The finding was interesting, with inhibition percentages at the corners ranging from 81 to 93% at concentrations ranging from 20 to 100 µg/mL Figure 3. The protective effect of three *Opuntia dillenii* oil seed varieties (AK, HA and IM) is significantly different (56.72 ± 3.97, 74.59 ± 4.23 and 91.56 ± 5.47 µg/mL) compared to the standard quercetin (71.41±2.11 µg/mL) (Table 3) at the doses used (20, 40, 60, 80 and 100 µg/mL). These findings show that samples of this plant have a high to moderate lipoxygenase inhibitory activity. The AK variety has the highest IC_{50} (56.72 ± 0.97 µg/mL), followed by HA (74.59 ± 0.23 µg/mL) and IM (91.56 ± 0.47 µg/mL).

Inhibition of Albumin Denaturation

Anti-inflammatory properties have also been evaluated using the inhibition of albumin denaturation. *In vitro*, The results show that all the samples (AK, HA and IM) tested at 20-100 µg/mL inhibit BSA denaturation with an 85% to 97%. The IM has the most potent inhibitory Effect (IC_{50}= 33.34±0.18 µg/mL). This inhibition is statistically similar to the reference Diclofenac (IC_{50} = 83.94± 1.53 µg/mL) at the same concentrations (Table 3).

Hypotonic solution-induced hemolysis of human red blood cells

From the results in Figure 3a and Table 4, the three oils of *O. dillenii* and concentration-dependently inhibited RBC membrane hemolysis in a hypotonic solution. The percentage inhibitions increased from 48.76, 66.16 to 74.31% at a concentration of 70, 100 and 250 mg/mL, respectively, for AKO, 36.18, 60,05 to 64.59% for HAO and 37.16, 50.99 to 57.14% at a same concentration for IMO. The percentage inhibition was comparable with diclofenac which was 48.88, 75.06 and 76.45% at concentrations of 70, 100 and 250 µg/mL, respectively, with an IC_{50} of 77.31 mg/mL, 79.58mg/mL and 80.25mg/mL.

Heat-induced hemolysis

The three *O. dillenii* oils also inhibited heat-induced hemolysis by 74.57, 72.76% and 59.12% at 250 mg/mL for KAO, HAO and IMO, respectively. The effect was similar to diclofenac which gave percentage inhibitions of 77.86% at concentrations of 250 mg/mL respectively, as shown in Figure 1b. and the IC_{50} was a range of 83.80 mg/mL 82.74 mg/mL 92.71 mg/mL compared to diclofenac as reference a (65.33 mg/mL).

The study and evaluation of the anti-inflammatory activity were carried out according to the *in vitro* method of stabilization of the RBC membrane, whose hemolysis was induced by two agents: heat or hypotonicity. The different oils of *O. dillenii* showed a considerable inhibitory effect against erythrocyte hemolysis induced by both heat and hypotonicity in a dose-dependent manner, probably explained by the ability of these oils to protect the membrane of rat red blood cells effectively.

O. dillenii is an excellent source of compounds with a wide range of biological activities, including anti-inflammatory properties. The abundance of phenolic compounds in oils, particularly flavonoids such as quercetin and kaempferol, tyrolsol and vanillic acid, may contribute to the anti-inflammatory effect demonstrated in this study [23-25]. Numerous studies have documented that plant-derived volatile compounds, whether present in substantial or minor quantities, exert diverse biological effects, including anti-inflammatory properties [26]. Nevertheless,

investigations have focused on the assessment of the anti-inflammatory potential of Monoterpene alcohols, including but not limited to linalool and linalyl acetate, as well as their corresponding esters. These compounds, recognized as prominent constituents among the volatile components of numerous aromatic plants, have exhibited considerable efficacy in combating inflammation, marking them as auspicious candidates within the realm of volatile bioactive agents [27].

For example, aliphatic and aromatic aldehydes have been shown to have significant anti-inflammatory and antimicrobial properties 101. There is a good correlation between anti-inflammatory activity and the terpenes and sesquiterpenes evaluated, which appear to be good 5-LOX inhibitors *in vitro.* Sesquiterpene alcohols, aliphatic aldehydes and some phenolic esters are phytochemicals that are known to have anti-inflammatory activities, namely cyclooxygenase (COX) and lipoxygenase (LOX) [28]. In our finding, the anti-inflammatory properties may be due to the presence of aliphatic aldehydes (trans-2-Decenal, Dodecanal and Decanal) and esters (Benzyl salicylate, Citronellyl acetate and Linalyl acetate) in *O. dillenii* oil seed of three varieties [28, 29]

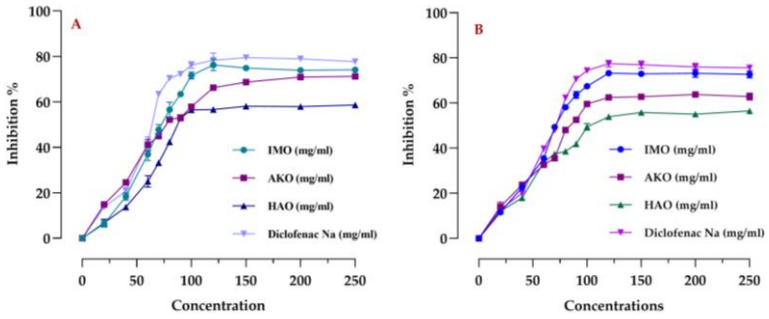


Figure 4. Inhibition percentage values obtained with Hypotonia (A) and Heat-induced hemolysis, (B) assay of *O. dillenii* oils

Table 4 . The IC_{50} values (mg/mL), obtained with Hypotonia and Heat-induced hemolysis assay, of *O. dillenii* oils

Samples/ Reference	Hypotonia	Heat
AK	83.80 ± 1.54	77.31 ± 0.63
HA	82.74 ± 0.29	79.58 ± 0.59
IM	92.71 ± 1.28	80.25 ± 1.38
Diclofenac Na (mg/mL)	65.33 ± 1.51	75.54 ± 0.64

Natural products. Particularly those of plant origin. It has been an exciting source of new compounds in the search for bioactive molecules widely used in therapeutics as antioxidant activity. Therefore, the antioxidant capacity of the oils extracted from the fruit of *O. dillenii* was evaluated. For this purpose. The following methods were studied: iron reduction (FRAP). 2.2-diphényl 1-picrylhydrazyle reduction (DPPH) as well as ferrous iron chelation capacity (FIC). The results differed significantly depending on the test chosen. The ability of extracts to neutralize DPPH varied significantly with concentration. Table 5 shows the results.

According to the findings, there is a proportional increase in DPPH radical scavenging activities as a function of extract concentrations. IM variety has the greatest antioxidant effect, followed by AK and HA. with IC_{50} values of 15.17±1.28. 38.41±1.54 and 42.24±0.29 µg/mL, respectively. Trolox has an IC_{50} value of 19.18±1.51 µg/mL as the reference standard.

Table 5. IC_{50} (µg/mL) value of the Antioxidant Activity

Samples/ Standards	Antioxidant Activity IC_{50} (µg/mL)		
	DPPH	FRAP	FIC
AK	38.41 ± 1.54	30.23 ± 0.60	42.75 ± 0.63
HA	42.24 ± 0.29	55.96 ± 0.08	39.54 ± 0.59
IM	15.17 ± 1.28	23.41 ± 1.83	35.31 ± 1.38
Trolox	19.18 ± 1.51	-	-
Gallic acid	-	39.29 ± 1.35	-
Ascorbic acid	-	-	47.21 ± 0.64

Generally, the reducing power of the ethanolic and aqueous extracts and that of the fractions increased with their concentrations. At a 1mg/mL dose. the IM and AK showed excellent antioxidant activity (IC_{50}: 23.41 ± 1.83, and 30.23 ± 0.60 µg/mL). respectively. Compared to gallic acid (39.29 ± 1.35µg/mL) taken as a reference. The HA has a minor effect compared to the others (55.96 ± 0.08 µg/mL). The reducing power is probably due to the presence of the hydroxyl group in the phenolic compounds, which can serve as an electron donor. Therefore. Antioxidants are considered to reduce and inactivate oxidants [30].

The chelating capacity of ferrous iron varied significantly between the samples studied (Table 23). The FIC of the AK. HA and IM were significantly higher (IC_{50}: 42.75 ± 0.63, 39.54 ± 0.59, and 35.31 ± 1.38 µg/mL) than the reference's IC_{50} (47.21 ± 0.64 µg/mL) (ascorbic acid). These differences could be due to differences in the phytochemical composition contained.

The antioxidant Effect of the different extracts of our plant could be due to a synergism between polyphenols and other components. In fact, the latter is known to be excellent antioxidants whose oxide-reducing properties allow them to act as reducing agents. Hydrogen donors and oxygen inhibitors [31] due to their chemical structures with many hydrogen atoms. Hydroxyl groups. Phenylated rings could capture free radicals by demobilizing their single electrons [32].

Toxicity prediction using Pro-Tox II

Table 6 provides important information regarding the toxicity predictions and toxic endpoints of major compounds found in *O. dilleni*. The compounds are listed from (1) catechin to (14) kaempferol. The table includes data on predicted LD50 values (lethal dose for 50% of the population) and hazard classes. The table also provides information on organ toxicity and various toxicity endpoints, such as hepatotoxicity, immunotoxicity, mutagenicity, and carcinotoxicity. The terms "Act." and "Ina." indicate whether a compound is predicted to be active or inactive, respectively, in causing the specific toxicity endpoints. The corresponding probabilities (labeled as "Prob.") offer insights into the likelihood of a compound exhibiting specific toxic effects.

Analyzing the table, the catechin, with a predicted LD50 of 10,000 mg/kg, falls under hazard class VI, indicating it is considered non-toxic. Similarly, vanillic acid, vanillin, and rutin, with LD50 values falling from 2,000 mg/kg to 5,000 mg/kg, are classified as hazard class V. This suggests that these compounds may be harmful if swallowed. Among the compounds categorized as hazard class IV (300 mg/kg < LD50 ≤ 2,000 mg/kg), the tyrosol, caffeic acid, ferulic acid, quercetin 3-O-β-D-glucoside, and others. These compounds are considered harmful if swallowed. They demonstrate a range of predicted LD50 values, indicating differing potential toxicity levels. Notably, quercetin, with a predicted LD50 of 159 mg/kg, falls under hazard class III. This classification suggests that quercetin is toxic if swallowed and requires caution.

Additionally, several compounds exhibit activity in specific toxicity endpoints. For example, compounds like caffeic acid, sinapic acid, ferulic acid, and rutin show activity in toxicity endpoints such as carcinogenicity and immunotoxicity. Their respective probability values indicate the likelihood of activity in these endpoints.

In the sample AKO, p-coumaric acid has been identified as an active carcinogenic agent with a moderate probability of 0.50. This suggests that there is a reasonable likelihood that p-coumaric acid may contribute to developing cancerous conditions. Furthermore, AKO and HAO contain sinapic and ferulic acids, albeit in varying quantities. These compounds have undergone screening and have been determined to have immunotoxic properties. This implies that sinapic acid and ferulic acid present in AKO and HAO may impact the immune system, potentially affecting its functioning and response. In addition to the immunotoxic effects, cinnamic acid in HAO is hepatotoxic. This indicates that cinnamic acid, when consumed or exposed to the liver, may potentially cause damage or toxicity to this particular organ.

10.1002/cbdv.202301890

It is essential to consider these findings as indicators of potential risks associated with the presence of these compounds. However, further investigation and research are necessary to fully understand the extent of their toxicity and determine safe levels of exposure or consumption.

10.1002/cbdv.202301890

Table 6. Prediction of toxicity and the toxic endpoints of the major compounds found in *O. dillenii*. (1) catechin, (2) 4-hydroxybenzoic acid, (3) tyrosol, (4) caffeic acid, (5) vanillic acid, (6) vanillin, (7) p-coumaric acid, (8) sinapic acid, (9) ferulic acid, (10) quercetin 3-o-β-d-glucoside, (11) rutin, (12) quercetin, (13) cinnamic acid, (14) kaempferol.

No.	Predicted LD_{50} (mg/kg)	Class	Organ toxicity				Toxicity endpoints					
			Hepatotoxicity		Carcinogenicity		Immunotoxicity		Mutagenicity		Carcinotoxicity	
			Predi.*	Prob.	Predi.	Prob.	Predi.	Prob.	Predi.	Prob.	Predi.	Prob.
1	10000	VI	Ina.	0.72	Ina.	0.51	Ina.	0.96	Ina.	0.55	Ina.	0.84
2	2200	V	Ina.	0.52	Ina.	0.51	Ina.	0.99	Ina.	0.99	Ina.	0.86
3	1030	IV	Ina.	0.82	Ina.	0.75	Ina.	0.99	Ina.	0.93	Ina.	0.91
4	2980	V	Ina.	0.57	Act.	0.78	Ina.	0.50	Ina.	0.98	Ina.	0.86
5	2000	IV	Ina.	0.55	Ina.	0.64	Ina.	0.97	Ina.	0.96	Ina.	0.93
6	1000	IV	Ina.	0.52	Ina.	0.60	Ina.	0.55	Ina.	0.98	Ina.	0.94
7	2850	V	Ina.	0.51	**Act.**	**0.50**	Ina.	0.91	Ina.	0.93	Ina.	0.81
8	1772	IV	Ina.	0.54	Ina.	0.67	**Act.**	**0.89**	Ina.	0.87	Ina.	0.96
9	1772	IV	Ina.	0.51	Ina.	0.61	**Act.**	**0.91**	Ina.	0.96	Ina.	0.88
10	1034	IV	**Act.**	**0.51**	Ina.	0.67	Ina.	0.99	Ina.	0.98	Ina.	0.86
11	5000	V	Ina.	0.80	Ina.	0.91	**Act.**	**0.98**	Ina.	0.88	Ina.	0.64
12	159	III	Ina.	0.69	**Act.**	**0.68**	Ina.	0.87	Ina.	0.51	Ina.	0.99
13	2500	V	**Act.**	**0.54**	Ina.	0.82	Ina.	0.95	Ina.	0.96	Ina.	0.83
14	3919	V	Ina.	0.68	Ina.	0.72	Ina.	0.96	Ina.	0.52	Ina.	0.98

10.1002/cbdv.202301890

To evaluate the results of the given antioxidant and anti-inflammatory activity and clarify the mechanisms of action of the bioactive compounds in *O. dillenii*. An *in-silico* study investigating the antioxidant and anti-inflammatory activities of *O. dillenii* phenolic compounds revealed significant effects against NADPH oxidase and lipoxygenase, respectively. Molecular docking has been used against both BSA and LOX receptors as a powerful bioinformatics tool. It allows an atomistic overview of the binding mode of each bioactive compound to the receptors active site. Thus providing crucial knowledge and understanding of these natural molecules effectiveness for their uses as therapeutic agents.

Regarding antioxidant activity, rutin and vanillic acid exhibited the highest potency against NADPH oxidase, with a glide Gscore of -6.989 Kcal/mol and -6.618 Kcal/mol, respectively.

Regarding anti-inflammatory activity, sinapic acid and kaempferol were the most effective molecules against lipoxygenase, yielding a glide Gscore of -7.065 Kcal/mol and -6.961 Kcal/mol, respectively. These compounds displayed notable potential in suppressing the inflammatory response (Table 7).

Compared to the standards, rutin, vanillic acid, catechin, and *p*-coumaric acid demonstrated greater inhibition energy against NADPH oxidase when compared to Trolox (-6.363 kcal/mol). Furthermore, all the molecules investigated for their inhibitory power against lipoxygenase exhibited higher inhibition energy than diclofenac (-5.595 kcal/mol), except for tyrosol (-3.867 kcal/mol) and quercetin 3-*O*-β-D-glucoside (-5.415 kcal/mol) (Table 7).

10.1002/cbdv.202301890

Table 7. Docking results in ligands in different receptors

	antioxidant activity			anti-inflammatory activity		
	Glide gscore Kcal/mol	**Glide emodel Kcal/mol**	**Glide energy Kcal/mol**	**Glide gscore Kcal/mol**	**Glide emodel Kcal/mol**	**Glide energy Kcal/mol**
Rutin	-6.989	-87.795	-61.324	-6.548	-99.305	-68.316
Vanillic acid	-6.618	-42.986	-27.939	-6.367	-43.475	-19.78
Catechin	-6.532	-53.928	-37.389	-5.681	-53.357	-36.181
***p*-Coumaric acid**	-6.073	-45.238	-29.611	-6.394	-41.106	-19.167
Caffeic acid	-5.988	-47.241	-30.903	-6.514	-44.596	-22.641
Sinapic acid	-5.981	-51.585	-34.694	-7.065	-50.399	-24.049
Ferulic acid	-5.49	-45.828	-30.8	-6.555	-45.554	-22.364
Vanillin	-6.427	-39.849	-25.406	-6.166	-32.705	-12.518
Tyrosol	-5.163	-31.951	-23.73	-3.867	-30.673	-23.111
4-Hydroxybenzoic acid	-5.102	-36.443	-24.239	-6.422	-41.624	-18.149
Cinnamic acid	-4.731	-36.026	-24.139	-5.802	-36.27	-17.496
Quercetin	-6.23	-68.339	-46.008	-6.707	-51.068	-27.781
Quercetin 3-*O*-β-D-glucoside	-5.734	-74.432	-51.96	-5.415	-70.344	-50.885
Kaempferol	-5.385	-57.673	-40.932	-6.961	-49.801	-25.845
Diclofenac	NA	NA	NA	-5.595	-51.953	-27.908
Trolox	-6.363	-53.064	-34.023	NA	NA	NA

NA: not applicable

10.1002/cbdv.202301890

found that rutin forms four hydrogen bonds within the active site of NADPH oxidase. These hydrogen bonds are established with specific residues, namely LYS187, ASP179, and VAL214. Rutin forms a Pi-cation bond with residue LYS213 in the same active site. In contrast, when interacting with the active site of NADPH oxidase, vanillic acid forms a single hydrogen bond with residue VAL214 and a salt bridge with residue LYS213 (Figure 4 and 5).

Moreover, sinapic acid established a salt bridge with the FE2 701 residue in the lipoxygenase active site. At the same time, kaempferol also established a single hydrogen bond with the GLU614 residue in the same active site (Figure 4 and 5).

10.1002/cbdv.202301890

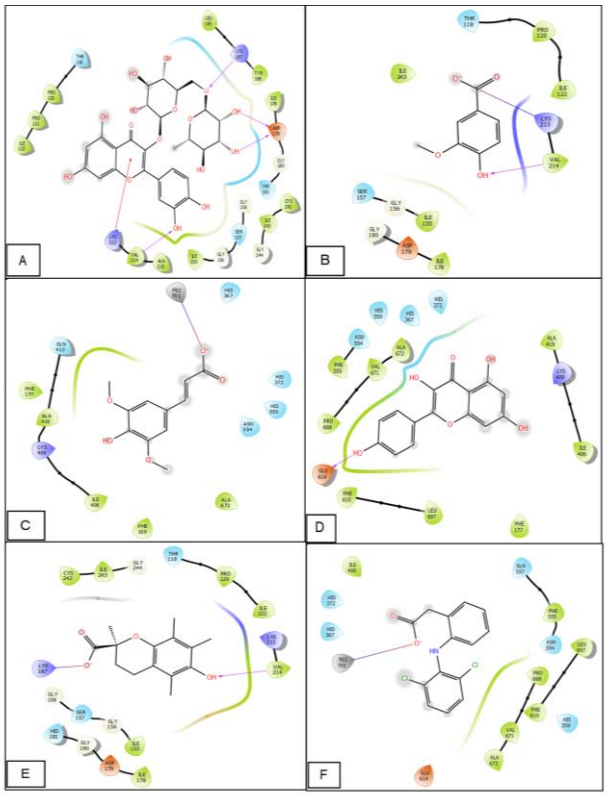
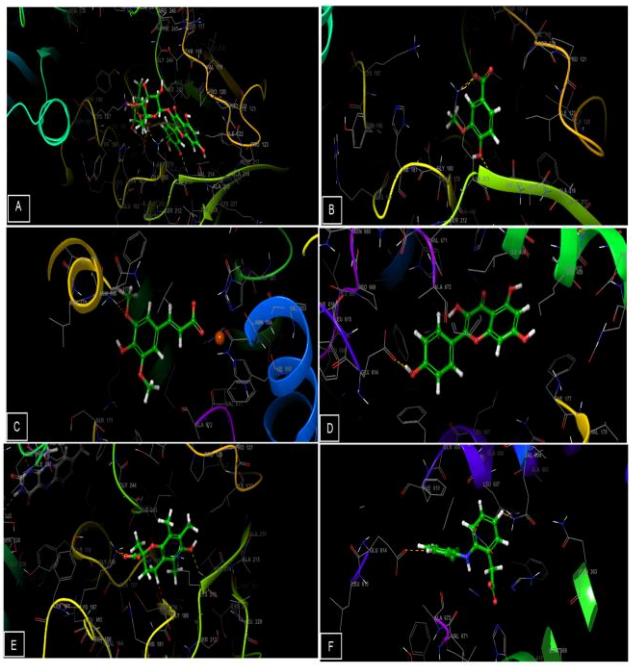


Figure 5. The 2D viewer of ligands interactions with the active site. A, B and E: rutin, vanillic acid and Trolox interactions with active site of NADPH oxidase. C, D, and F: sinapic acid, kaempferol and Diclofenac interactions with active site of Lipoxygenase

10.1002/cbdv.202301890

408



409

Figure 6. The 3D viewer of ligands interactions with the active site. A, B and E: rutin , vanillic acid and Trolox interactions with active site of NADPH oxidase. C, D, and F: sinapic acid, kaempferol and Diclofenac interactions with active site of lipoxygenase

410
411
412

413

10.1002/cbdv.202301890

Conclusions

Opuntia dillenii, sometimes known as prickly pear or nopal, is a plant that contains chemical compounds that have biological function. Bioactive chemicals have a variety of health effects and are frequently studied in pharmaceutical research. The study aims to investigate the phytochemical screening, anti-inflammatory and antioxidant potentials of *Opuntia dillenii* oil seeds. The oils had anti-inflammatory activity as determined by BSA, LOX, Hypotonia and Heat assay. Additionally, the results indicated that *Opuntia dillenii* oil has potent antioxidant properties. In general, results indicated that these oils possess potent bioactivities. The phytochemical screening showed that *Opuntia dillenii* oil contains a substantial amount of phenolic composition. Quercetin 3-O-β-D-glucoside was the most prevalent component found in AKO and IMO cultivars, with concentrations of 25.74 and 38.92 mg/100g, respectively. While it was identified in a modest amount in the AKO variety (2.95 mg/100g), it was followed by vanillic acid (25.59 mg/100g) in IMO and it exists in a limited amount in the AKO and HAO varieties with levels of 1.70 and 3.35 mg/100g, respectively. The presence of those compounds in the samples is responsible for pharmacology activity. GC-MS analyses have substantiated the presence of noteworthy quantities of volatile constituents within *O. dillenii*. Aldehydes have previously been recognized as a component of the scent of *O. dillenii*, and aldehydes and alcohols were found in significant concentrations in *Opuntia* oil and fruit (HA, AK, and IM). Aldehydes were the most common type of volatile organic compound (VOC), accounting for 57.33 0.24%, 68.25 0.7%, and 55.50 0.11% of total VOCs in HA, IM, and AK Opuntia pulp, and 48.92 0.7%, 64.7 0.11%, and 62.52 0.2% in HA, IM, and AK Opuntia oil. The potential involvement of E-11-hexadecenal and (E)-2-undecenal in the demonstrated *in vitro* anti-inflammatory and antioxidant efficacy is evident. This ascertained data furnishes scientific substantiation for the established traditional therapeutic applications of *Opuntia dillenii* while concurrently underscoring a propitious avenue for the advancement of anti-inflammatory pharmaceutical agents derived from this botanical source.

In summary, the research appears to center on *Opuntia dillenii*, studying its bioactive characteristics and volatile profiles in depth, with a particular focus on its possible pharmaceutical applications. This type of research is widespread in the subject of pharmacognosy, which is concerned with the investigation of natural compounds as potential sources of novel medications.

Experimental Section

Plant materials

In this study, three Moroccan *Opuntia* fruit varieties were used: Harmoucha (HA) from Oujda, Aknari (AK) from Nador (Northern Morocco), and Imtchan (IM). The matured purple fruits of prickly pear (*O. dillenii*) were collected in March 2023 from Essaouira.

Fresh species were collected in sterile plastic bags and systematically brought to the laboratory. Fresh fruit seeds were separated. The separated seeds were allowed to dry at room temperature before being cold-pressed with an oil extraction machine. Extracted seed oil and fresh pulp were packaged in a 30 mL glass bottle and stored at -4°C until use.

Cold-pressed extraction

The raw material seeds are mechanically pressed to extract the necessary oils in cold-pressed extraction. To avoid heat-induced damage to the sensitive molecules, the materials are crushed or ground at moderate temperatures, usually below 120°F (49°C).The seeds are placed between permeable barriers, increasing the mechanical pressure and reducing the volume available for the seeds.

In general, whatever the seeds used, the higher the pressure, the more efficient the oil extraction. In a p55/AFYACH oil press voltage 220V/380V electrical power 1.5 kw, serial number 0020200107, we put 3 kg of *Opuntia* seeds in the press, an extraction that lasts around 45 minutes, then we filter our oil through a 20-plate mechanical filter and store at 4°C until use [33].

Phenolic compound extraction

The phenol extraction was carried out in a liquid/liquid manner. One gram of oil was combined with two milliliters of 99% methanol, vortexed for ten minutes, and centrifuged at 4500 rpm for fifteen minutes. The supernatant was pipetted into a volumetric flask, and each sample was extracted three times. The final volume collected was 5 mL and stored at 4°C. On the same day as the extraction, the extract was examined using HPLC equipment.

Headspace Procedure

Before the isolation of volatile compounds, 1 gram of the chosen plant material (vegetal oil and Fresh pulp) was carefully weighed into weighing boats. Subsequently, it was transferred into 20 mL vials using a clamp and sealed within those vials until it reached room temperature for frozen samples. The Solid-Phase Microextraction (SPME) fibers were exposed to the headspace of these samples for 10 minutes at 80°C. Following this, the extracted analytes were desorbed, separated, and promptly identified using Gas Chromatography-Mass Spectrometry (GC-MS).

GC-MS analysis

The prepared samples (1 µL) were injected into an automatic injection system using a Shimadzu GC system (Kyoto, Japan) equipped with a BPX25 capillary column with a 5% diphenyl and 95% dimethylpolysiloxane phase (30 m, 0.25 mm, and 0.25 m) and coupled to a QP2010 MS. The mobile phase was helium gas (99.99%) at a flow rate of 1.69 L/min. The injection, ion source, and interface temperatures were set to 250 ◦C, while the temperature program for the column oven was set to 50 ◦C for 1 min before being heated to 250 ◦C at a rate of 10 ◦C/min and maintained for an additional minute. The sample components were ionized in the Electron Ionization (EI) mode at 70 eV and a scanned mass range of 40 to 300 m/z. The NIST mass spectral library of the CG-MS data system was used to compare the results [34-36]. The peak area was used to compute the percentage composition.

Determination of phenolic compounds by HPLC-DAD

Separation of phenolic compounds was performed on an Agilent 1200 (Agilent Technologies, Palo Alto, CA, USA) linked to a diode array UV detector (Bruker, Germany). Each extract (20 µL) was injected into a Zorbax XDB-C18 (5 µm porosity, 250 4.6 mm; Agilent Technologies series 1100 system (Palo Alto, CA, USA)) column and an elution gradient of 0-25 min, 20% B; 25-30 min, 100% B; and 30-35 min, 20% B. The mobile phases employed for sample elution were A (water/0.5% phosphoric acid) and B (methanol), with a flow rate of 1 mL/min. The separation was carried out at a constant temperature of 40°C. At 254, 280, 320, 350, and 540, spectrophotometric detection was done, as described in our works [37, 38].

Antioxidant activity

DPPH assay

The assessment of free radical scavenging activity was conducted via spectrophotometry following the procedure outlined by Elbouzidi et al., [39]. Specifically, 100 µL of the extract was introduced into 1 mL of the DPPH solution. Subsequently, the reduction in absorbance was determined at 517 nm following a 30-minute incubation in a dark environment. Ascorbic acid served as the standard for comparison. The computation of the percentage of antioxidant activity was carried out using the following formula **(1)**:

$$I\,\% = ((A_{control} - A_{Sample}) / A_{control}) \times 100 \quad \textbf{(1)}$$

Where:

$A_{control}$: Absorbance of the control after 30 minutes.

A_{Sample}: Corresponding the Absorbance of extract.

Ferric-reducing power assay (FRAP)

The assessment of ferric-reducing power (FRAP) was conducted following the protocol outlined by Oyaizu et al., [40]. In this procedure, 250 µL of the sample was combined with 250 µL of phosphate buffer (0.2 M, pH 6.6), and 250 µL of a 1% potassium ferricyanide solution ($K_3Fe(CN)_6$). After a 20-minute incubation at 50°C, the mixture was supplemented with 250 µL of trichloroacetic acid (10%), 1 mL of distilled water, and 250 µL of ferric chloride (0.1%). The resulting absorbance was then measured at 700 nm. Ascorbic acid served as the standard, and the reducing power was expressed as a percentage.

The percentage calculation was determined using the following formula **(2)**:

$$I\ \% = ((A_{control} - A_{Sample}) / A_{control}) \times 100 \quad \textbf{(2)}$$

Where:

$A_{control}$: Absorbance of the control after 30 minutes.

A_{Sample}: Absorbance of the test corresponding to our extract.

Ferrous Iron Chelating (FIC) Assay

The ability of the extracts to chelate iron (II) was determined according to the method described by Dinis et al., [41] with some modifications. In summary, 100 µL of the extract was added to 50 µL of $FeCl_2$ (2.0 mM) and 1.8 mL of distilled water. After 5 minutes, the reaction was initiated by adding 50 µL of ferrozine (5.0 mM). After incubation for 10 minutes, the absorbance was measured at 562 nm.

Anti-inflammatory activity

Lipoxygenase inhibition test

The evaluation of the anti-inflammatory attributes in the extracts adhered to the protocol elucidated by Nikhila et al. [42]. In this methodology, a reaction concoction was formulated, encompassing 200 µL of the extract at diverse concentrations (100, 75, 50, and 25 µg/mL in methanol), 35 µL of lipoxygenase (Sigma, Darmstadt, Germany) at a concentration of 0.1 mg/mL, and 600 µL of a 0.2 M borate buffer solution with a pH of 9.0. Subsequently, this amalgamation was subjected to an incubation period of 15 minutes at a temperature of 25 °C. The induction of the reaction was initiated by the introduction of 35 µL of a substrate solution (comprising 250 µL linoleic acid), and the ensuing absorbance was gauged at 234 nm. The percentage calculation was determined using the following formula **(3):**

$$\text{Denaturation inhibition percentage } \% = (Abs_{Blank} - Abs_{Sample}) / (Abs_{Blank}) \times 100 \quad \textbf{(3)}$$

Abs_{blank} is the absorbance of the reaction medium without extract, and **Abs_{sample}** is the absorbance of the reaction medium with extract.

Inhibition of the albumin denaturation test

The work was performed following the method described by Kar et al., [43]. The mixture comprised 50 µL of the extract, meticulously prepared at distinct concentrations (100, 75, 50, and 25 µg/mL in methanol), along with a volume of 450 µL of a 2% solution of Bovine Serum Albumin (BSA) and 2.5 mL of a phosphate-buffered saline solution with a pH of 6.3, adjusted through the addition of hydrochloric acid (HCl). The tubes housing the mixture underwent an incubation period of 15 minutes at a temperature of 37 °C, followed by a subsequent heating stage at 57 °C for 5 minutes, and culminating in a cooling phase for 10 minutes [44]. The spectrophotometer was employed to ascertain the absorbance of these solutions at a wavelength of 660 nm. The experimentation was conducted in triplicate. Diclofenac was utilized as the reference standard, formulated at an equivalent concentration to the extracts. The quantification of the albumin denaturation inhibition, presented as a percentage in relation to the control, was computed utilizing the subsequent expression **(4)**:

$$\text{Denaturation inhibition percentage } \% = (Abs_{Blank} - Abs_{Sample}) / (Abs_{Blank}) \times 100 \quad \textbf{(4)}$$

where **Abs_{blank}** is the absorbance of the reaction medium without extract, and **Abs_{sample}** is the absorbance of the reaction medium with extract.

Red Blood Cell (RBCs) Membrane Stabilization Test

The anti-inflammatory properties of *O. dillenii* specimens were evaluated through the employment of the RBC methodology. Wistar rats within the weight range of 300-350g were subjected to ether-induced anesthesia, and subsequently, fresh blood was procured via catheterization from the abdominal aorta. This collected blood was then placed into glass tubes containing a homogeneous blend of a sterilized anticoagulant solution comprising 2% dextrose, 0.8% sodium citrate, 0.5% citric acid, and 0.42% sodium chloride. Erythrocyte cell concentrates were subjected to purification with sterile saline (0.9% w/v NaCl, pH 7.2) until the resulting supernatant attained clarity. The volumes of the cellular pellet were measured, following which a 10% suspension was formed by combining the cellular pellet with a sterilized sodium phosphate buffer solution (adjusted to pH 7.4) at a concentration of 10 mM. The composition of the sodium phosphate buffer solution was as follows: 0.2 g NaH_2PO_4, 1.15 g Na_2HPO_4, and 9 g NaCl, formulated in a volume of 1 L of distilled water. The reconstituted red blood cells were employed in heat or hypotonicity-induced hemolysis assays. The percentage of hemolysis was calculated under the assumption that the control yielded 100% hemolysis.

Erythrocyte Hypotonia-Induced Hemolysis

Various concentrations of *O. dillenii* extracts were prepared by dissolving them in distilled water. To achieve this, 0.1 mL of a 10% erythrocyte suspension was combined, resulting in a final volume of 5.1 mL. Diclofenac sodium, an established anti-inflammatory drug, served as the reference substance. The reaction mixtures were allowed to incubate for a duration of 1 hour at a temperature of 37°C. Subsequently, centrifugation was conducted at 3500 rpm for a duration of 15 minutes. The quantification of hemoglobin content present in the supernatant was executed using a spectrophotometer set to a wavelength of 540nm. The experimental procedure was carried out in triplicate, and the resulting mean values were subsequently computed.

Erythrocyte Heat-Induced Hemolysis

Various concentrations of *O. dillenii* were formulated using an isotonic solution buffered with phosphate (0.15M, pH 7.4). To achieve this, 0.1mL of a 10% suspension of erythrocytes was added, resulting in a final volume of 5.1mL. A comparative control was established with 5mL of the carrier solution (isotonic phosphate buffer), while a standard tube containing 5mL of diclofenac sodium (a reference anti-inflammatory drug) at diverse concentrations was prepared using the same buffer.

After preparation, all reaction mixtures were subjected to an incubation period at 60°C for a duration of 30 minutes. Following this, the tubes were allowed to cool to ambient temperature. Ultimately, centrifugation at a rate of 3000 revolutions per minute (rpm) for 10 minutes was conducted. The quantification of hemoglobin content was performed on the resulting supernatant solution utilizing a spectrophotometer set at a wavelength of 540nm. The experimental trials were executed in triplicate, and the mean values were subsequently computed. The following expression was used to determine the inhibition percentage of hemolysis induced by hypotonicity/Heat **(5)**:

$$\textbf{\% Inhibition} = ((DO_{control} - DO_{Sample})/DO_{control})) \times 100 \quad \textbf{(5)}$$

DO control measures the optical density of the heated/hypotonia control solution. At the same time, DO Sample measures the optical density of the heated/hypotonia sample solution.

Toxicity Prediction Using Pro-Tox II

The Protox II online tool (https://tox-new.charite.de/protox II/, accessed on 21st June 2023) was employed to assess toxicity levels. This tool provided information on LD_{50} values, toxicity class, and various toxicological endpoints [45]. Using these approaches and equipment, important findings were obtained concerning the potential therapeutic uses and negative impacts of the main chemical compounds found in HA, AK and IM.

Molecular Docking

Preparation of ligands

The phenolic constituents present in *O. dillenii*, identified utilizing High-Performance Liquid Chromatography with Diode Array Detection (HPLC-DAD), were procured from PUBCHEM in Structure-Data File (SDF) format. To prepare the ligands for the docking simulations, the LigPrep utility integrated within the Schrödinger Software suite (version 11.5) was harnessed, leveraging the OPLS3 force field. A total of 32 stereoisomeric configurations were generated for each ligand, considering ionization states within the pH range of 7.0 ± 2.0 [46].

Preparation of Proteins

In the study of antioxidant activity, the crystal structures of NADPH oxidase were obtained from the protein data bank with PDB ID: 2CDU, whereas lipoxygenase (PDB ID: 3V99) was used to study the anti-inflammatory activity. The Protein Preparation Wizard in Schrödinger-Maestro (protein preparation) program further refined and prepared the structures. All water molecules were removed, hydrogens bonded to heavy atoms, methionines replaced selenomethionines, and charges and bond orders were subsequently assigned. The OPLS3 force field was utilized to minimize, resulting in a maximum heavy atom RMSD of 0.30 Å [47].

Generation of Receptor's Grid

An atom originating from the ligand molecule was selected as the focal point, and a default three-dimensional grid enclosure was generated. This grid enclosure was characterized by equidistant spatial intervals, each measuring 20 units along the x, y, and z axes, respectively, and was employed for two distinct receptors. The precise spatial coordinates of the grid enclosure were defined as follows: x: 19.853, y: -6.431, z: -0.896 for NADPH oxidase, and x: 18.342, y: -78.663, z: -33.95 for lipoxygenase. Utilizing the 'standard Precision' (SP) methodology, the ligand was affixed to the grid enclosure aligned with the protein structure. Subsequent evaluation of the outcomes was conducted through the application of the XP GScore metric.

Glide Standard Precision (SP) Ligand Docking

SP flexible ligand docking was carried out in the glide of Schrödinger-Maestro v 11.5. Penalties were enforced for amide bonds exhibiting non-cis/trans conformations. The parameters governing the scaling factor for van der Waals interactions and the threshold for partial charge on ligand atoms were established at 0.80 and 0.15, respectively. The conclusive evaluation involved energy-minimized orientations, and the outcomes were assessed utilizing the Glide scoring metric. The optimal docking arrangement for each ligand was pinpointed and recorded, representing the pose associated with the minimum Glide score value [46, 48].

Declaration of conflict of interest: The authors declare there is no conflict of interest regarding the publication of this paper.

Keywords: Antioxidant and Anti-inflammatory Activity; Oil; *Opuntia dillenii*, Phenolic Compounds, Volatile Compounds.

References

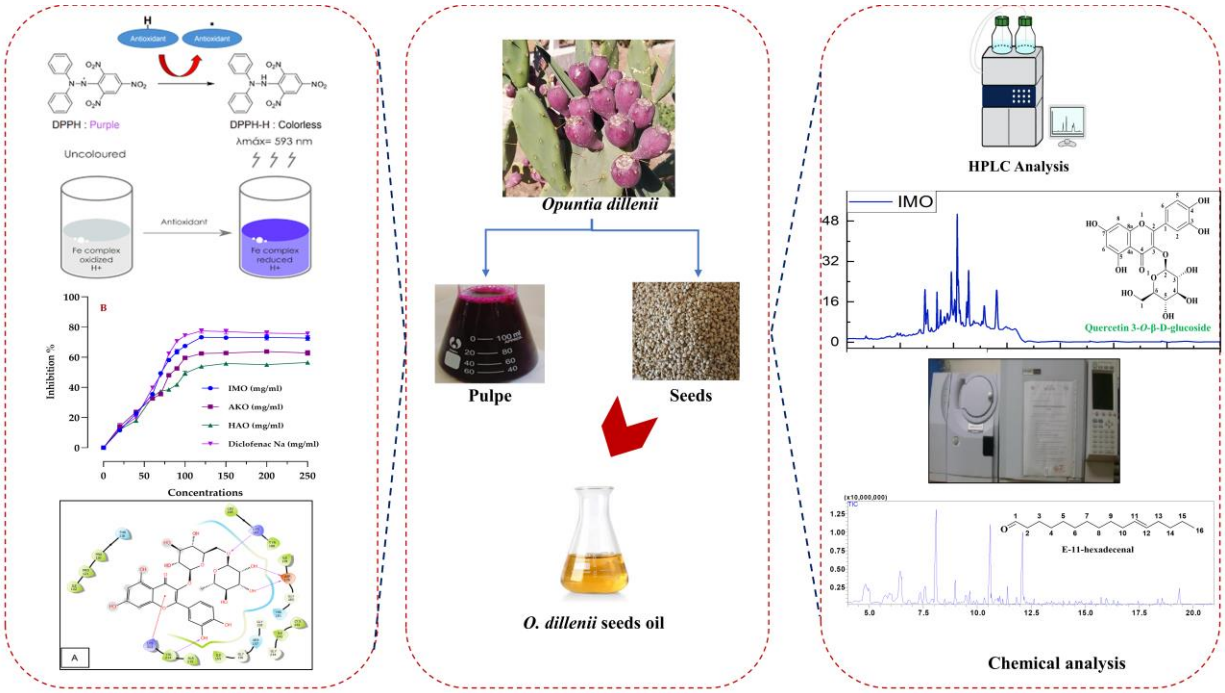
1. Hirasa, K.; Takemasa, M., *Spice science and technology*. CRC Press: 1998.
2. Karmakar, I.; Dolai, N.; Saha, P.; Sarkar, N.; Bala, A.; Haldar, P. K., Scavenging activity of Curcuma caesia rhizome against reactive oxygen and nitrogen species. *Orient Pharm Exp Med* **2011,** 11, (4), 221-228.
3. Scalbert, A., Fruits et légumes, polyphénols et santé. *Laboratoire des maladies métaboliques et micronutriments, INRA* **2004**.
4. Visioli, F.; Romani, A.; Mulinacci, N.; Zarini, S.; Conte, D.; Vincieri, F. F.; Galli, C., Antioxidant and other biological activities of olive mill waste waters. *J. Agric. Food Chem* **1999,** 47, (8), 3397-3401.
5. Chougui, N.; Tamendjari, A.; Hamidj, W.; Hallal, S.; Barras, A.; Richard, T.; Larbat, R., Oil composition and characterisation of phenolic compounds of Opuntia ficus-indica seeds. **2013,** 139, (1-4), 796-803.
6. Kaur, M.; Kaur, A.; Sharma, R., Pharmacological actions of Opuntia ficus indica: A Review. *J. Appl. Pharm. Sci* **2012,** 2, (7), 15-18.
7. Bouhrim, M.; Ouassou. H.; Choukri. M.; Mekhfi. H.; Ziyyat. A.; Legssyer. A.; Bnouham, M., Hepatoprotective effect of *Opuntia dillenii* seed oil on CCl_4 induced acute liver damage in rat. *Asian Pac. J. Trop. Biomed.* **2018,** 8, (5), 254.
8. Chahdoura, H.; Barreira, J. C.; Fernández-Ruiz, V.; Morales, P.; Calhelha, R. C.; Flamini, G.; Soković, M.; Ferreira, I. C.; Achour, L., Bioactivity, proximate, mineral and volatile profiles along the flowering stages of Opuntia microdasys (Lehm.): defining potential applications. **2016,** 7, (3), 1458-1467.
9. Schwab, W.; Davidovich‐Rikanati, R.; Lewinsohn, E., Biosynthesis of plant‐derived flavor compounds. *Plant J* **2008,** 54, (4), 712-732.
10. Hjelmeland, A. K.; Ebeler, S. E., Glycosidically bound volatile aroma compounds in grapes and wine: a review. *Am. J. Enol. Vitic* **2015,** 66, (1), 1-11.
11. de Ancos, B.; Ibanez, E.; Reglero, G.; Cano, M. P., Frozen storage effects on anthocyanins and volatile compounds of raspberry fruit. *J. Agric. Food Chem* **2000,** 48, (3), 873-879.
12. Khalil, M. N.; Fekry, M. I.; Farag, M. A., Metabolome based volatiles profiling in 13 date palm fruit varieties from Egypt via SPME GC–MS and chemometrics. *Food Chem* **2017,** 217, 171-181.
13. Defilippi, B. G.; Manríquez, D.; Luengwilai, K.; González-Agüero, M., Aroma volatiles: biosynthesis and mechanisms of modulation during fruit ripening. *Adv. Bot. Res* **2009,** 50, 1-37.
14. Chahdoura, H.; Barreira, J. C.; Barros, L.; Dias, M. I.; Calhelha, R. C.; Flamini, G.; Soković, M.; Achour, L.; Ferreira, I. C., Bioactivity, hydrophilic, lipophilic and volatile compounds in pulps and skins of Opuntia macrorhiza and Opuntia microdasys fruits. **2019,** 105, 57-65.
15. Zito, P.; Sajeva, M.; Bruno, M.; Rosselli, S.; Maggio, A.; Senatore, F., Essential oils composition of two Sicilian cultivars of Opuntia ficus-indica (L.) Mill.(Cactaceae) fruits (prickly pear). *Nat. Prod. Res* **2013,** 27, (14), 1305-1314.
16. Al-Naqeb, G.; Cafarella, C.; Aprea, E.; Ferrentino, G.; Gasparini, A.; Buzzanca, C.; Micalizzi, G.; Dugo, P.; Mondello, L.; Rigano, F., Supercritical Fluid Extraction of Oils from Cactus Opuntia ficus-indica L. and Opuntia dillenii Seeds. **2023,** 12, (3), 618.
17. EL Hassania, L.; Btissam, B.; Mohamed , B.; Farid , A.; Manon, G.; Kahina, Z.; Mohamed, B.; Mohammed, B.; Belkheir, H.; Mohamed, A.; Mohammed, R.; Marie-Laure, F., Chemical Composition, Antibacterial, Antifungal and An-tidiabetic Activities of Ethanolic extracts of *Opuntia dillenii* Fruits Collected from Morocco. *J. Food Qual* **2022**, 15.
18. Katanić, J.; Yousfi, F.; Caruso, M. C.; Matić, S.; Monti, D. M.; Loukili, E. H.; Boroja, T., F., ; Mihailović, V.; Galgano, F.; Imbimbo, P.; Petruk, G.; Bouhrim, M.; Bnouham, M.; Ramdani, M., Characterization of bioactivity and phytochemical composition with toxicity studies of different *Opuntia dillenii* extracts from Morocco. *Food Biosci* **2019,** 30, 100410.
19. Ali, B.; Abderrahim, Z.; Hassane, M.; Marianne, S.; Marie-Laure, F.; Abdelkhaleq, L.; Mohammed, A.; Mohamed, B., Chemical Composition of Cactus Pear Seed Oil: phenolics identification and antioxidant activity. **2022,** 25, (2), 121.
20. Chougui, N.; Tamendjari. A.; Hamidj. W.; Hallal. S.; Barras. A.; Richard. T.; Larbat, R., Oil composition and characterisation of phenolic compounds of Opuntia ficus-indica seeds. **2013,** 139, (1-4), 796-803.
21. Jick, H., Risk of upper gastrointestinal bleeding and perforation associated with individual non-steroidal anti-inflammatory drugs. *The Lancet* **1994,** 343, (8900), 769-772.
22. Barnes, P. J., Anti-inflammatory actions of glucocorticoids: molecular mechanisms. **1998,** 94, (6), 557-572.
23. Yoon, C.-H.; Chung, S.-J.; Lee, S.-W.; Park, Y.-B.; Lee, S.-K.; Park, M.-C., L'acide gallique, acide polyphénolique naturel, induit l'apoptose et inhibe l'expression des gènes pro-inflammatoires dans les synoviocytes fibroblastiques de polyarthrite rhumatoïde. *Revue du rhumatisme* **2013,** 80, (3), 271-278.

24. Park, M.-Y.; Kwon, H.-J.; Sung, M.-K., Evaluation of aloin and aloe-emodin as anti-inflammatory agents in aloe by using murine macrophages. *Biosci. Biotechnol. Biochem* **2009,** 73, (4), 828-832.
25. Gupta, M.; Bhalla, T.; Gupta, G.; Mitra, C.; Bhargava, K., Anti-inflammatory activity of taxifolin. *JPN J pharmacol* **1971,** 21, (3), 377-382.
26. García-Lafuente, A.; Guillamón, E.; Villares, A.; Rostagno, M. A.; Martínez, J. A., Flavonoids as anti-inflammatory agents: implications in cancer and cardiovascular disease. *J. Inflamm. Res* **2009,** 58, (9), 537-552.
27. Pasdaran, A.; Sheikhi, D., Volatile oils: Potential agents for the treatment of respiratory infections. *Mic Resp ses infec* **2016**, 237-261.
28. Baylac, S.; Racine, P., Inhibition of 5-lipoxygenase by essential oils and other natural fragrant extracts. **2003,** 13, (2-3), 138-142.
29. Perera, H. D. S. M.; Samarasekera, J. K. R. R.; Handunnetti, S. M.; Weerasena, O. V. D. S. J., In vitro anti-inflammatory and anti-oxidant activities of Sri Lankan medicinal plants. *Ind Crops Prod* **2016,** 94, 610-620.
30. Siddhuraju, P.; Becker, K., The antioxidant and free radical scavenging activities of processed cowpea (Vigna unguiculata (L.) Walp.) seed extracts. *Food Chem* **2007,** 101, (1), 10-19.
31. Pietta, P.-G., Flavonoids as antioxidants. *J. Nat. Prod* **2000,** 63, (7), 1035-1042.
32. Calliste, C.-A.; Trouillas. P.; Allais. D-P.; Simon. A.; Duroux, J.-L., Free radical scavenging activities measured by electron spin resonance spectroscopy and B16 cell antiproliferative behaviors of seven plants. **2001,** 49, (7), 3321-3327.
33. Kadda, S.; Belabed, A.; Loukili, E. H.; Hammouti, B.; Fadlaoui, S., Temperature and extraction methods effects on yields, fatty acids, and tocopherols of prickly pear (Opuntia ficus-indica L.) seed oil of eastern region of Morocco. *Environ. Sci. Pollut. Res* **2022**, 1-9.
34. Haddou, S.; Loukili, E. H.; Hbika, A.; Chahine, A.; Hammouti, B., Phytochemical study using HPLC-UV/GC–MS of different of Cannabis sativa L seeds extracts from Morocco. *Mater. Today: Proc.* **2023,** 72, 3896-3903.
35. Loukili, E. H.; Abrigach, F.; Bouhrim, M.; Bnouham, M.; Fauconnier, M.-l.; Ramdani, M., Chemical composition and physicochemical analysis of Opuntia dillenii extracts grown in Morocco. *J Chem* **2021,** 2021, 1-11.
36. Loukili, E. H.; Ouahabi, S.; Elbouzidi, A.; Taibi, M.; Yahyaoui, M. I.; Asehraou, A.; Azougay, A.; Saleh, A.; Al Kamaly, O.; Parvez, M. K., Phytochemical Composition and Pharmacological Activities of Three Essential Oils Collected from Eastern Morocco (Origanum compactum, Salvia officinalis, and Syzygium aromaticum): A Comparative Study. **2023,** 12, (19), 3376.
37. Laaroussi, H.; Aouniti, A.; Hafez, B.; Mokhtari, O.; Sheikh, R.; Hamdani, I.; Rahhou, I.; Loukili, E. H.; Belbachir, C.; Hammouti, B., Argan leaves aqueous extract's antioxidant activity and mild steel corrosion inhibition ability. **2022,** 11, (4), 1539-1556.
38. Haddou, S.; Elrherabi, A.; Loukili, E. H.; Abdnim, R.; Hbika, A.; Bouhrim, M.; Al Kamaly, O.; Saleh, A.; Shahat, A. A.; Bnouham, M., Chemical Analysis of the Antihyperglycemic, and Pancreatic α-Amylase, Lipase, and Intestinal α-Glucosidase Inhibitory Activities of Cannabis sativa L. Seed Extracts. *Molecules* **2023,** 29, (1), 93.
39. Elbouzidi, A.; Taibi, M.; Ouassou, H.; Ouahhoud, S.; Ou-Yahia, D.; Loukili, E. H.; Aherkou, M.; Mansouri, F.; Bencheikh, N.; Laaraj, S., Exploring the Multi-Faceted Potential of Carob (Ceratonia siliqua var. Rahma) Leaves from Morocco: A Comprehensive Analysis of Polyphenols Profile, Antimicrobial Activity, Cytotoxicity against Breast Cancer Cell Lines, and Genotoxicity. **2023,** 16, (6), 840.
40. Oyaizu, M., Studies on products of browning reaction. *JJ Nut Diet* **1986,** 44, (6), 307-315.
41. Dinis, T. C.; Madeira, V. M.; Almeida, L. M., Action of phenolic derivatives (acetaminophen, salicylate, and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. *Arch. Biochem. Biophys* **1994,** 315, (1), 161-169.
42. Nikhila, G.; Sangeetha, G., Anti inflammatory properties of the root tubers of *Gloriosa superba* and its conservation through micropropagation. *J. Med. Plant Res* **2015,** 9, (1), 1-7.
43. Kar, B.; Kumar, R. S.; Karmakar, I.; Dola, N.; Bala, A.; Mazumder, U. K.; Hadar, P. K., Antioxidant and in vitro anti-inflammatory activities of Mimusops elengi leaves. *Asian Pac. J. Trop. Biomed* **2012,** 2, (2), S976-S980.
44. Nea, F.; Tanoh, E. A.; Wognin, E. L.; Kemene, T. K.; Genva, M.; Saive, M.; Tonzibo, Z. F.; Fauconnier, M.-L., A new chemotype of Lantana rhodesiensis Moldenke essential oil from Côte d'Ivoire: Chemical composition and biological activities. *nd Crops Prod* **2019,** 141, 111766.
45. Banerjee, P.; Eckert, A. O.; Schrey, A. K.; Preissner, R., ProTox-II: a webserver for the prediction of toxicity of chemicals. **2018,** 46, (W1), W257-W263.

46. Aboul-Soud, M. A.; Ennaji, H.; Kumar, A.; Alfhili, M. A.; Bari, A.; Ahamed, M.; Chebaibi, M.; Bourhia, M.; Khallouki, F.; Alghamdi, K. M., Antioxidant, anti-proliferative activity and chemical fingerprinting of centaurea calcitrapa against breast cancer cells and molecular docking of caspase-3. **2022,** 11, (8), 1514.
47. Amrati, F. E.-Z.; Slighoua, M.; Mssillou, I.; Chebaibi, M.; Galvão de Azevedo, R.; Boukhira, S.; Moslova, K.; Al Kamaly, O.; Saleh, A.; Correa de Oliveira, A., Lipids Fraction from Caralluma europaea (Guss.): MicroTOF and HPLC Analyses and Exploration of Its Antioxidant, Cytotoxic, Anti-Inflammatory, and Wound Healing Effects. **2023,** 10, (3), 172.
48. Ouahabi, S.; Loukili, E. H.; Daoudi, N. E.; Chebaibi, M.; Ramdani, M.; Rahhou, I.; Bnouham, M.; Fauconnier, M.-L.; Hammouti, B.; Rhazi, L., Study of the Phytochemical Composition, Antioxidant Properties, and In Vitro Anti-Diabetic Efficacy of Gracilaria bursa-pastoris Extracts. *Mar. Drugs* **2023,** 21, (7), 372.

10.1002/cbdv.202301890

Graphical abstract



Twitter Text: Analyzing the Bioactive Properties and Volatile Profiles Characteristics of *Opuntia dillenii* (Ker Gawl.) Haw: Exploring its Potential for Pharmacological Applications *by* El Hassania et al., @GkhanZe75178190