



Volatile compounds and bioactivities properties of *Opuntia microdasys* (Lehm.)

Hassiba Chahdoura¹, Asma El-Ayeb-Zakhama², Mejdi Snoussi³, Guido Flamini^{4,5}, Manel Ben M'hadheb¹, Safia El-Bok^{6*} & Lotfi Achour²

¹Unité de Recherche "Génomique, Biotechnologie et Stratégies Antivirales", Institut Supérieur de Biotechnologie, Université de Monastir, BP74, Avenue Tahar Hadded, 5000 Monastir, Tunisia.

²Laboratoire de Recherche "Bioressources, Biologie Intégrative & Valorisation", Institut Supérieur de Biotechnologie de Monastir, Tunisia.

³Department of Biology, College of Science, University of Hail, Hail P.O. Box 2440, Saudi Arabia.

⁴Dipartimento di Farmacia, via Bonanno 6, 56126 Pisa, Italy.

⁵Centro Interdipartimentale di Ricerca "Nutraceutica e Alimentazione per laSalute" Nutra food, University of Pisa, Italy.

⁶Laboratoire de Biodiversité, Biotechnologies & Changements Climatiques, Université Tunis El-Manar, Département de Biologie, Faculté des Sciences de Tunis, Campus Universitaire, 2092 Tunis, Tunisia.

Article info

Article history:

Received: 28 December 2023

Accepted: 26 February 2024

Keywords: *Opuntia microdasys* Lehm; *Opuntia macrorhiza* Engelm; cladodes; HS-SPME-GC/MS; Phytochemical compound; Anatomy.



Copyright©2024 JOASD

*Corresponding author

safia1elbok@yahoo.fr

Conflict of Interest: The authors declare no conflict of interest.

Abstract

The seeds of *Opuntia* sp. require additional investigations because they could contain several active molecules. Therefore, the present study analyzed the chemical composition and biological activities of *Opuntia microdasys* and that of *Opuntia macrorhiza* using *in vitro* and *in vivo* models.

The headspace of the powders of the seeds of *Opuntia* have been analyzed by gas chromatography–mass spectrometry (GC–MS). The analgesic activity was assessed using the acetic acid-induced abdominal constriction response on mouse. Chemical analyses showed that the powders of the seeds of *O. microdasys* and *O. macrorhiza* were rich in nonanal and camphor. Also, the *O. macrorhiza* seeds has a high antioxidant activity (EC₅₀ =0.12 mg/ml). The results showed that the ethanolic extracts, at a dose of 200 mg/kg of body weight, caused a significant ($P < 0.05$) inhibition of the abdominal constriction response (75.17% and 56.22% for *O. microdasys* and *O. microdasys*). The microdilution assay revealed that ethanolic extract of *O. macrorhiza* seeds is more active and could inhibit the growth of almost all the evaluated bacterial and fungal strains, with the minimal bactericidal concentration values (MIC) ranging from 0.039 to 2.50 mg/mL.

The overall results highlighted the potential of the tested *Opuntia* seeds that can be used as a source of bioactive compounds that confer various important biological activities to it, which could imply other pharmacological applications.

1. INTRODUCTION

Opuntia (Cactaceae, Opuntioideae) is native to arid and semiarid regions. This genus includes about 300 different species, considering both domesticated and wild species (FAO, 2002; Melga et al., 2017). Cactaceae, a native New World plant family, are typically found in a vast territory, which extends from British Columbia and Alberta in Canada to Patagonia in Argentina (Galvez et al., 2012). Cactus pear grows throughout Tunisia and the fruits are consumed exclusively as fresh fruits (Ennouri et al.,2006).

The amount of seeds is important as it varies from 20 to 40% dry-weight of the whole fruit, depending on the cultivars (Habibi et al., 2005). The literature contains much information concerning the juice and cladode of the cactus pear, but studies on the chemical composition and biological activity of seeds are scarce (El Kossori et al.,1998 ; Coskuner et al.,2003; Morales et al.,2012; Chougui et al., 2013; Chahdoura et al., 2015b; Chahdoura et al.,2018, Bouaouich et al., 2023). All the authors agree about the richness of the *Opuntia ficus-indica*

seed oil in polyunsaturated fatty acids and vitamins. These features may potentially permit its inclusion in animal and human diets (Ennouri et al., 2006). Chahdoura et al. (2015) reported that methanolic extracts of *Opuntia microdasys* and *macrorhiza* seeds possessed notable antioxidant activity and antitumoral inhibitory effect. Their results also demonstrated that the higher amounts of polyphenols and flavonoids in the seeds of the two species of *Opuntia* may contribute to the stronger antioxidant activity of the seeds. The seeds are rich in oil, and the high amount of unsaturated fatty acids may also possess potentially notable antioxidant activity (Kolniak-Ostek et al., 2020).

Opuntia has also been studied as a source of fibers, minerals, proteins and amino acids (Majdoub et al., 2001 and Chahdoura et al., 2015a).

In this context, the present study aims to analyze the aromatic compounds and antioxidant and analgesic activities of the seeds of *O. microdasys* and *O. macrorhiza*.

2. MATERIAL AND METHODS

2.1. Plant collection

Fresh fruit samples consisted of two species of *Opuntia* (*O. microdasys* and *O. macrorhiza*) collected from the Cliff of Monastir, Tunisia in 2022. The plant was identified by Professor Fethia Harzallah Skhiri (High Institute of Biotechnology of Monastir, Tunisia). These fruits were hand-picked, washed and processed manually for the separation of seeds from the whole fruit. The seeds were lyophilized and were further processed by powdering with a commercial food blender and stored at - 20 °C until analysis.

2.2. Volatile compounds

Supelco (Bellefonte, PA) SPME devices coated with polydimethylsiloxane (PDMS, 100 µm) were used to sample the head-space of dry seeds inserted into a 5 mL vial and allowed to equilibrate for 30 min. After the equilibration time, the fibre was exposed to the headspace for 50 min at room temperature. Once sampling was finished, the fibre was withdrawn into the needle and transported to the injection port of the GC-MS system. All the SPME sampling and desorption conditions were identical for all the samples. Furthermore, blanks were performed before each first SPME extraction and randomly repeated during each series. Quantitative comparisons of relative peaks areas were

performed between the same chemicals in the different samples. GC-EIMS analyses were executed with a Varian (Palo Alto, CA) CP3800 gas chromatograph equipped with a DB-5 capillary column (30 m - 0.25 mm - 0.25 µm; Agilent) and a Varian Saturn 2000 ion trap mass detector. The analytical settings were as follows: injector and transfer line temperatures were 250 and 240°C, respectively; oven temperature was programmed from 60 to 240°C at 3 °C/min; carrier gas was helium at 1 mL/min; splitless injection. Constituents identification was based on a comparison of the retention times with those of authentic samples, comparing their linear retention indices and on computer matching against the commercial (NIST 2014 and Adams 2007) and homemade library of mass spectra, and MS literature data.

2.3. Determination of the total anthocyanins

An amount (0.25g) of powder was extracted in the dark with 10 mL of acidified methanol (1% HCl), kept for 30 min at 37°C and then centrifuged for 15 min. The results were expressed as µg cyanidin - 3 -glucoside per g dry weight (Strack and Wary, 1989).

2.4. Extract preparation

For ethanol/water (80:20, v/v) extraction, fifty grams of powdered seeds were extracted twice for 2 h in a magnetic stirrer plate (25° C at 250 rpm), with ethanol/water (80:20, v/v), then filtered through a Whatman No. 4 paper and vacuum-dried in a rotary evaporator at 40° C to remove the solvent. The extracts were further frozen and lyophilized. Afterwards, the extracts were re-dissolved in ethanol/water (80:20, v/v) for *in vitro* and *in vivo* activity assays.

2.5. Determination of bioactive components

Phenolic and *O*-diphenols compounds in the hydrolic extracts were estimated by a colorimetric assay, based on procedures described by Montedoro et al., (1992). Results were expressed as milligrams of gallic acid equivalents (GAEs) per gram of extract. Flavonoid contents in the extracts were determined by a colorimetric method described by Jia et al., (1999) with some modifications.

2.6. Antioxydant activity

2.6.1. Ferrous ions chelating assay

The iron-chelating capability of the two extracts of seeds was tested according to the method of Zhu et al., (2006), with minor modifications. For each test, 100 µL of seeds extract, dissolved in

ethanol/water at different concentrations, were added to 50 μ L of FeSO₄ (2 mmol/L). The obtained mixtures were incubated for 5 min at room temperature and the reaction was triggered by the addition of 100 μ L of ferrozine solution (5 mmol/L). The mixtures were incubated at room temperature (10 min). The absorbance of the final solutions was measured at 562 nm, and the chelating activity (%) was calculated. The concentration providing 50% of radical scavenging activity (EC₅₀) was calculated from the graph of inhibition percentage against seeds extract concentration; EDTA was used as a reference compound.

2.6.2. Phosphomolybdenum assay

An aliquot (200 μ L) of ethanol extract of seeds was mixed with 2 mL of the phosphomolybdenum reagent (0.6 M sulfuric acid, 28 mM sodium phosphate, 4 mM ammonium molybdate) (Prieto et al.,1999). The test tubes were incubated for 90 min at 95 °C and then cooled to room temperature. Subsequently, the absorbance was measured at 695 nm. A standard curve was constructed using ascorbic acid. EC₅₀ (mg/mL) is the effective concentration at which the total antioxidant activity was 50% and was obtained by interpolation from linear regression analysis.

2.7. *In vitro* assessment of antimicrobial activity

2.7.1. Test strains

The antimicrobial activity of the tested extracts was assessed against a panel of microorganisms including five each Gram positive and Gram-negative bacteria and four *Candida* strains.

2.7.2. Micro-well determination of MIC and MBC/MFC

The determination of the minimal inhibition concentrations (MIC) and the minimal bactericidal/fungicidal concentrations (MBC/MFC) were performed by the technique of dilution using 96-well plates, as previously described by (Snoussi et al.,2018). First, the different samples were dissolved in 10% DMSO and diluted with culture broth to the concentration of 10 mg.mL⁻¹. Then, the serial dilutions were achieved from well 2 to well 9 by addition the desired concentrations ranging from 10 to 0.039 mg.mL⁻¹ in 5 mL sterile glass tubes containing nutrient broth. 5 μ L of each microbial suspension were added to these wells by aseptically transferring. The first well containing the nutrient Sabouraud

Chloramphenicol without product and 5 μ L of the inoculum was exploited as a negative control. The micro-plates were incubated at 37 °C for approximately 24 h and these studies were performed in triplicate. For the reading, the minimum inhibitory concentration (MIC) was definite as the minimum concentration of the compounds to inhibit the growth of the microorganisms. MBC and MFC values were defined as the lowest concentration that showed no microorganisms growth in the subcultures.

2.8. Animals

Male mice (24–28 g) were used for the experimentations. Animals were maintained on 12 h light/dark rotation at about 25 \pm 1° C, with relative humidity of 60 – 70% and free access to diet and water. All animals were adapted to the new environment minimum two weeks before initiating the studies. All experiments were carried out using six animals in each respective group. The animals were handled according to the guidelines of the Tunisian Society for the Care and Use of Laboratory Animals (ATSAL), and the protocol for the rat studies was approved by Institutional Animal Ethics Committee for Animal Care and Use, Bizerte University, Carthage.

2.9. Analgesic test

Acetic acid-induced abdominal constriction response in Swiss albino mice was evaluated by dividing the animals into seven groups (n = 6). One group, pretreated subcutaneously (s.c.) with 10 mL/kg of saline, served as control; the second group was s.c. pretreated with the reference drug (ASL) at the dose of 200 mg/kg 30 min before intraperitoneal administration (i.p.) of 1% acetic acid at the dose of 10 mL/kg. The remaining groups were i.p. injected with 10 mL/kg of 1% acetic acid solution 30 min after the s.c. administration of the crude extract at the doses of 50, 100 and 200 mg/kg. The number of writhing was recorded during 30 min after the acetic acid injection. A writhe is indicated by abdominal constriction and stretching of at least one hind limb. Analgesic activity was expressed as inhibition percent of the usual number of writhes observed in control animals (Koster et al., 1959).

3. Statistical Analysis

All the assays of aromatic compounds were performed in triplicate. Data are expressed as mean values and standard deviation (SD). The differences between the different samples were

analysed using one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference post hoc of Dunnett's test with $\alpha = 0.05$. This treatment was carried out using SPSS v. 18.0 program.

3. RESULTS AND DISCUSSION

3.1. Volatile compounds

The chemical composition of the headspace of the powders obtained from the dry seeds of *O. microdasys* and *O. macrorhiza* are presented in Table 1. The seeds of *O. microdasys* revealed the presence of eight components, representing 91.20% of the total volatiles. The major constituents of were camphor (40.80%), nonanal (29.80%) and limonene (3.00%). Four compounds were identified for the seeds of *O. macrorhiza*, representing 98.10% of the total volatiles. The main components, even if in different percentages, were again camphor (71.70%), nonanal (20.80%) and limonene (2.90%).

Limonene is commonly used as a fragrance ingredient in numerous consumer products (Hansen et al., 2016). The application of limonene, especially in aromatherapy, has expanded significantly, but its potential effects

on cellular metabolism should be further studied. It has been observed that limonene directly binds to the adenosine A2A receptor, which may induce sedative effects. Limonene also increased cytosolic calcium concentration, which can be achieved by the activation of adenosine A2A receptors. These findings suggest that limonene can act as a ligand and an agonist for adenosine A2A receptors (Park et al., 2011).

3.2. Phytochemical analysis

Total polyhenols, flavonoids, flavonol, tannins and O-diphenols, contents in the seeds of *Opuntia* sp. grown in Tunisia are shown in Table 2. The seeds of *O. macrorhiza* shows high levels of TPC of the order of 55.34 and 39.84 mg EAG / g of extract for *O. macrorhiza* and *O. microdasys*, respectively (Table2). Significant differences ($p < 0.05$) were also found between the two varieties of *Opuntia* sp. Previous studies have also reported that the total phenolic content of *Opuntia* sp. ranged from 221.3 to 612.10 g GAE/100 g DM (Abou-Elella and Ali, 2014; Shima et al., 2022). Moreover, identified compounds such as quercetin, kaempferol and rutin possess antiproliferative and antioxidant activities (Chahdoura et al., 2015).

Table 1. Volatile compounds (%) of *Opuntia microdasys* and *O. macrorhiza* seeds.

Constituents	L.R.I	G1	G2
Octanal	1003	2.90±0.02	-
Limonene	1032	3.00 ^a ±0.01	2.90 ^b ±0.02
(E)-2-octenal	1063	2.70±0.04	-
Nonanal	1104	29.80 ^b ±0.02	20.80 ^a ±0.05
Camphor	1146	40.80 ^a ±1.08	71.70 ^b ±1.33
Decanal	1206	8.60 ^b ±0.05	2.70 ^a ±0.01
n-Tridecane	1300	1.80±0.02	-
n-Tetradecane	1400	1.60±0.01	-
Monoterpene hydrocarbons		3.00 ^a ±0.01	2.90 ^a ±0.01
Oxygenated monoterpenes		40.80 ^a ±0.07	71.70 ^b ±1.33
Non-terpene derivatives		47.40 ^b ±0.05	23.50 ^a ±0.02
Total identified		91.20	98.10

LRI: linear retention indices on DB-5 column. G1: *O. microdasys* seeds; G2: *O. macrorhiza* seeds. Data expressed as means ± SD of three independent analyses (n = 3). Values in a line followed by the same letter are not significantly different at P < 0.05 (LSD test).

Table 2. Bioactive compound contents of the hydroalcoholic extracts of *Opuntia microdasys* and *O. macrorhiza* seeds (mean ± SD; n=3).

Parametres	G1	G2
Phenols (mg/g)	26.09 ^a ±1.08	55.34 ^b ±0.64
<i>O</i> -diphenols (mg/g)	11.25 ^a ±1.08	29.09 ^b ±1.02
Flavonoids (mg/g)	12.74 ^a ±0.27	54.60 ^b ±0.09
Flavonols (mg/g)	7.25 ^a ±0.08	12.33 ^b ±1.02
Tannins (mg/g)	9.60 ^a ±0.08	20.09 ^b ±0.05
Anthocyanins (µg/g)	5.08 ^a ±0.021	11.57 ^b ±0.01

G1: *O. microdasys* seeds; G2: *O. macrorhiza* ; dw: dry weight. Values in a line followed by the same letter are not significantly different at $P < 0.05$ (LSD test).

3.4. Antioxydant activity

As shown in Table 3, the antioxidant activity of the hydroalcoholic extract of *O. microdasys* and *O. macrorhiza* was measured spectrophotometrically using the phosphomolybdenum and ferrous ions chelating methods. The results are expressed as the EC₅₀ (lower EC₅₀ values indicate higher antioxidant activity). Overall, seeds of *O. macrorhiza* (EC₅₀= 0.12 and 0.68 mg/ml for phosphomolibdum and iron-chelating capability assays, respectively) were associated with a lower EC₅₀ than seeds of *O. microdasys*. Indeed, previous studies have shown that the reducing power of a component can serve as a significant indicator of its potential antioxidant activity (Kumaran et Karunakaran, 2007). Variation in the antioxidant capacity of these extracts may be attributable to differences in the phenols content (Chahdoura et al., 2015).

3.5. Antimicrobial activity

The results of the microdilution method revealed that the lowest MIC values are recorded with

O. macrorhiza seeds were obtained against *Bacillus cereus* (0.039 mg/ml) and *Staphylococcus aureus* (0.312 mg/ml). The MIC values of the fungal strains ranged from 2.50 to 10 mg/mL (Table 4).

Majority of previous studies showed that *Opuntia* genus have a great antifungal potential, thanks to their wealth of oxygenated monoterpene and particularly phenol compounds (Benramdane et al., 2022). Flavonoids are known for a wide range of actions, including antimicrobial activity (Ozçelik et al., 2011). The study by Shimaa et al., (2022) showed significant antimicrobial activity of *Opuntia* seed against *Bacillus cereus*, *Listeria monocytogenes*, *Staphylococcus aureus*, *E. Coli*. In a global view, the Gram-positive class was more sensitive to the lipophilic extracts used than the Gram-negative one, evidence that was already reported in several studies (Palmeri et al., 2020).

3.6. Analgesic activity

In the acetic acid induced writhing method, all the tested hydroalcoholic extracts of seeds significantly exhibited a dose-dependent

Table 3. Antioxydant properties of the hydroalcoholic extracts of *Opuntia microdasys* and *O. macrorhiza* seeds.

Antioxydant activity (EC ₅₀ values mg/mL)	G1	G2
Phosphomolybdenum assay	1.01 ^b ±0.01	0.12 ^a ±0.01
Ferrous chelating activity	1.56 ^b ±0.03	0.68 ^a ±0.02

Values in a line followed by the same letter are not significantly different at $P < 0.05$ (LSD test). G1: *O. microdasys* seeds; G2: *O. macrorhiza* seeds.

Table 4. Antibacterial and antifungal activities of *Opuntia microdasys* and *macrorhiza* seeds extract.

Microorganisms	G1		G2		Ampicillin	
	MIC ^a	MBC ^b	MIC ^a	MBC ^b	MIC ^a	MBC ^b
Bacteria strains						
<i>Staphylococcus aureus</i> ATCC25923	1.25	2.5	0.312	0.625	0.08	0.625
<i>Bacillus cereus</i> ATCC 11778	2.50	5.00	0.039	0.078	0.08	0.625
<i>Listeria monocytogenes</i> CECT933	2.50	5.00	1.25	2.50	0.08	3.00
<i>Pseudomonas aeruginosa</i> PAO1	1.25	2.50	1.25	>10	0.1	12.00
<i>Escherichia coli</i> ATCC35218	2.5	5.00	2.50	5.00	0.02	3.00
<i>Salmonella enterica</i> subsp. <i>Enterica</i> ECT443	2.50	5.00	2.50	5.00	0.2	3.00
Yeast strains						
	G1	G2	Amphotericin B			
	MIC ^a	MFC ^c	MIC ^a	MFC ^c	MIC ^a	MFC ^c
<i>Candida parapsilosis</i> ATCC 20019	10.00	>10	2.50	10	0.2	0.39
<i>Candida albicans</i> ATCC 2019	10.00	>10	5.00	10	0.026	0.82
<i>Candida krusei</i> ATCC 6258	10.00	>10	10.00	>10	0.1	0.2
<i>Candida tropicalis</i> 06-85	10.00	>10	5.00	>10	0.42	6.75

*Ampicillin (10 mg/ml) or Amphotericin B (10 mg/ml).*a: Minimal Inhibitory Concentration (mg/ml). b: Minimal Bactericidal Concentration (mg/ml). c: Minimal Fungicidal Concentration (mg/ml).

reduction in the number of writhes when compared to control (Table 5). Acetic acid-induced vascular permeability is a typical capillary permeability assay in mouse model (Antonisamy et al., 2011). As shown in Table 5, the hydroalcoholic extract of *O. macrorhiza* at 200 mg/ml had an inhibitory action (75.17%) against peritoneal capillary permeability brought by acetic acid induction in mice model and, more important, than the reference drug ASL (200 mg/kg, 63.59% of inhibition). Camphor has a well-known history as antiseptic, analgesic,

counter irritant and rubefacient (Strickley, 2004). Moreover, based on previous studies done on a few species of *Opuntia* sp., we can suggest that the chemical composition of these extracts is consistent with that of related species. However, the seeds of our species are rich in PUFAs according to the study by Chahdoura et al. (2014) and in essential fatty acids. These compounds may be involved in the treatment of certain pathologies such as inflammation and pain.

Table 5. Effects of the hydroalcoholic extracts of *Opuntia microdasys* and *O. macrorhiza* seeds on acetic acid-induced writhing in mice.

Groups	Concentration (mg/kg)	Number of writhes	Inhibition of writhing (%)
Control	-	70.50±0.27	-
G1	50	44.50±1.64***	36.88
	100	34.83±0.96***	50.59
	200	30.83±0.81**	56.26
G2	50	35.00±0.32***	50.35
	100	27.50±0.53***	60.99
Reference drug (ASL)	100	17.50±0.55**	75.17
	200	25.66±0.37**	63.59

Values are expressed as mean ±S.E.M. (n= 6); ASL: Acetylsalicylate of lysine. G1: *Opuntia microdasys* seeds; G2: *Opuntia macrorhiza* seeds.

** $p \leq 0.05$ significant versus negative control by post hoc Dunnett's test.

*** $p \leq 0.05$ significant versus positive control by post hoc Dunnett's test.

4. Conclusion

It can be concluded that the hydroalcoholic extracts of the seeds of *O. microdasys* and *O. macrorhiza* have a significant antioxidant activity and both central and peripheral analgesic properties. The studied showed high antibacterial and antifungal activities against a wide range of microorganisms known to cause serious infections. This might be related to its chemical profile. However, further studies are needed to prove the results of the study and it is also recommended to conduct a thorough analysis of biosafety and mechanism of action using animal models.

REFERENCES

- Abou-Elella, F.M. , Ali, R.F.M. (2014). Antioxidant and anticancer activities of different constituents extracted from Egyptian prickly pear Cactus (*Opuntia ficus-indica*) Peel. *Biochem. Analytical Biochemistry* 3, 158.
- Adams, R.P. (2007). Identification of essential oil components by gas chromatography-mass spectroscopy, 4th Edition Carol Stream, IL: Allured Publishing Company.
- Antonisamy, P., Duraipandiyar, V., Ignacimuthu, S. (2011). Anti-inflammatory, analgesic and antipyretic effects of friedelin isolated from *Azima tetraacantha* Lam. in mouse and rat models. *Journal of Pharmacy and Pharmacology* 63, 1070-1077.
- Bouaouich, A., Bouguerche, F., Mahiaoui, H., Gregorio, P., Bendif, H. (2023). Phytochemical Elucidation and Antioxidant Activity of Seeds from Three Prickly Pear (*Opuntia ficus-indica* L.) Cultivars from Algeria. *Applied Science* 13(3), 1444.
- Chahdoura, H., Barreira, J.C.M. , Barros, L. , Santos-Buelga, C. , Ferreira, I.C.F.R, Achour, L.(2014). Phytochemical characterization and antioxidant activity of the cladodes of *Opuntia macrorhiza* (Engelm.) and *Opuntia microdasys* (Lehm.). *Food and Fonction* 5,2190.
- Chahdoura, H., Morales, P., Barreira, J.C.M. , Barros, L., Fernández-Ruiz, V., Ferreira, I.C.F.R, Achour, L. (2015a). Dietary fiber, mineral elements profile and macronutrients composition in different edible parts of *Opuntia microdasys* (Lehm.) Pfeiff and *Opuntia macrorhiza* (Engelm.). *LWT - Food Science and Technology* 64, 446-451.
- Chahdoura, H., Barreira, J. C.M., Barros, L., Santos-Buelga C., Ferreira, I. C.F.R., Achour, L. (2015 b). Seeds of *Opuntia* spp. as a novel high potential by product: Phytochemical characterization and antioxidant activity. *Industrial Crops and Products* 65, 383-389.

- Chahdoura, H., Chaouch, M. A., Chouchéne, W., Chahed, A., Achour, S., Adouni, K., Mosbah, H., Majdoub, H., Flamini, G., Achour, L. (2018). Incorporation of *Opuntia macrorhiza* Engelm. in cake-making: Physical and sensory characteristics. *LWT - Food Science and Technology* 90, 15-21.
- Chougui, N., Louaileche, H., Mohedeb, S., Mouloudj, Y., Hammoui, Y., & Tamendjari, A. (2013). Physico-chemical characterisation and antioxidant activity of some *Opuntia ficus-indica* varieties grown in north Algeria. *African Journal of Biotechnology* 12, 299-307.
- Coskuner, Y., & Tekin, A. (2003). Seed composition of prickly pear fruits. *Journal of the Science of Food and Agriculture* 83, 846-849.
- El Kossori, R. L., Villaume, C., El Boustani, E., Sauvaire, Y., & Méjean, L. (1998). Composition of pulp, skin and seeds of prickly pears fruit (*Opuntia ficus indica* sp.). *Plant Foods for Human Nutrition*, 52, 263-270.
- Galvez, M.J., Prat, M. I., Villamil, C. B. (2012). Immunochemical properties of seed proteins as systematic markers in *Cactaceae*. *Biochemical Systematics and Ecology* 44, 20-26.
- Gomez, A., Bozari, S., Yanmis, D., Gulluce, M., Sahin, F., Agar, G.(2015).Chemical composition and antibacterial activity of essential oils of two species of *Lamiaceae* against phytopathogenic bacteria. *Polish Journal of Microbiology* 64, 121-127.
- Habibi, Y., Mahrouz, M., Vignon, M. R. (2005). Arabinan-rich polysaccharides isolated and characterized from the endosperm of the seed of *Opuntia ficus-indica* prickly pear fruits. *Carbohydrate Polymers* 60, 319-329.
- Hansen, J.S., Nørgaard, A.W., Koponen, I.K., Sørli, J.B., Paidi, M.D., Hansen, S.W., Clausen, P.A., Nielsen, G.D., Wolkoff, P., Larsen, S.T. (2016). Limonene and its ozone-initiated reaction products attenuate allergic lung inflammation in mice. *Journal of Immunotoxicology* 13, 793-803.
- Jia, Z., Tang, M., Wu, J. (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chemistry* 64, 555-559.
- Kolniak-Ostek, J., Kita, A., Miedzianka, J., Andreu-Coll, L., Legua, P. and Hernandez, F. (2020). Characterization of Bioactive Compounds of *Opuntia ficus-indica* (L.) Mill. Seeds from Spanish Cultivars. *Molecules* 25(23), 5734.
- Kumaran, A., Karunakaran, R.J. (2007). *In vitro* antioxidant activities of methanol extracts of five *Phyllanthus* species from India. *Lebensmittel-Wissenschaft und Technologie* 40, 344-352.
- Majdoub, H., Roudesli, S., Picton, L., Le Cerf, D., Muller, G., & Grisel, M. (2001). Prickly pear nopals pectin from *Opuntia ficus indica* physico-chemical study in dilute and semi-dilute solutions. *Carbohydrate Polymers* 46, 69-79.
- Montedoro, G., Servili, M., Baldioli, M., Miniati, E. (1992). Simple and hydrolyzable phenolic compounds in virgin olive oil. Their extraction, separation, and quantitative and semiquantitative evaluation by HPLC. *Journal of Agricultural and Food Chemistry*, 40, 1571-1576.
- Morales, P., Ramírez-Moreno, E., Sanchez-Mata, M. D. C., Carvalho, A. M., & Ferreira, I. C. F. R. (2012). Nutritional and antioxidant properties of pulp and seeds of two xoconostle cultivars (*Opuntia joconostle* F.A.C. Weber ex Diguét and *Opuntia matudae* Scheinvar) of high consumption in Mexico. *Food Research International* 46(1).
- Park, H. M., Lee, J. H., Yaoyao, J., Jun, H. J., Lee, S. J. (2011). Limonene, a natural cyclic terpene, is an agonistic ligand for adenosine A_{2A} receptors. *Biochemical and Biophysical Research Communications* 404, 345-348.
- FAO. (2002). *Opuntia* spp. A strategic fodder and efficient tool for combat desertification in the Wana region. Cactus (*Opuntia* spp.) as forage. FAO plant production and protection paper. 169.
- Melgara, B., Pereira, E., Oliveira, M. B. P.P., Garcia-Castello, E. M., Rodriguez-Lopez, A. D., Sokovic, M., Lillian, B., Ferreira, I. C.F.R.(2017). Extensive profiling of three varieties of *Opuntia* spp. fruit for innovative food ingredients. *Food Research International* 101,259-265.
- Ozçelik, B., Kartal, M., Orhan, I.(2011). Cytotoxicity, antiviral and antimicrobial activities of alkaloids, flavonoids, and phenolic acids. *Pharmaceutical Biology* 49 (4), 396-402.
- Palmeri, R., Parafati, L., Arena, E., Grasseno, E., Restuccia, C., Fallico, B.(2020). Antioxidant and Antimicrobial Properties of Semi Processed Frozen Prickly Pear Juice as Affected by Cultivar and Harvest Time. *Foods* 9,235.
- Prieto, P., Pineda, M., Aguilar, M. (1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Analytical Biochemistry* 269, 337-341.

- Shimaa, K. A., Sara, M. M., El-Masry, S. S., Alkhalifah, D. H., Hozzein, W.N., Aboel-Ainin, M. (2022). Phytochemical screening and characterization of the antioxidant, anti-proliferative and antibacterial effects of different extracts of *Opuntia ficus-indica* Peel. *Journal of King Saud University - Science* 34 (7), 102216.
- Koster, R., Anderson, M., Beer, E.J. (1959). Acetic acid for analgesic screening, *Federation Proceedings journal* 18, 412-416.
- Snoussi, M., Noumi, E., Devasya, P.R., Trabelsi, N., Kanekar, S., F. Nazzaro, Fratianni, F., Flamini, G., De Feo, V., Al-Sieni, A. (2018). Antioxidant properties and anti-quorum sensing potential of *Carum copticum* essential oil and phenolics against *Chromobacterium violaceum*. *Journal of Food Science and Technology* 55 (8), 2824-2832.
- Strack, D. and Wary, V. (1989). Anthocyanins. In: *Methods in Plant Biochemistry*, Dey, P.M. and J.B. Harborne (Eds.), Academic Press, New York 1, 325-356.
- Strickley, R.G. (2004). Solubilizing Excipients in Oral and Injectable Formulations. *Pharmaceutical Research* 21 (2), 201-230.
- Zhu, L., Glahn, R.P., Yeung, C.K., Miller, D.D. (2006). Iron uptake by Caco-2 cells from Na Fe EDTA and FeSO₄: Effects of ascorbic acid, pH, and a Fe (II) chelating agent. *Journal of Agricultural and Food Chemistry* 54, 7924-7928.