

ELUCIDATION OF THE PHYSIOCHEMICAL AND PHYTOCHEMICAL PROPERTIES OF THE ETHANOLIC EXTRACT OF *Opuntia ficus-indica* CLADODES FROM VARIOUS REGIONS IN ALGERIA

Fatima Zohra EL MAHI¹, Fatima Zohra ELKADI¹, Habib MELIANI²,
MD Mahedi Hassan TUSHER³, Aicha RIH¹, Warda ZITOUNI¹, Meriem MAHI¹,
Abir MAKNI¹, Khadra NEMMICH¹

¹Laboratory of Molecular Microbiology Health and Proteomics, Biology Department, Faculty of Natural Sciences and Life, Djillali Liabés University of Sidi Bel Abbés, Algeria

²Eco-Development of Spaces Research Laboratory, Faculty of Natural and Life Sciences, Djillali Liabes University, Sidi Bel Abbes, Algeria

³Department of Pharmacology, Faculty of Basic Sciences, Bangladesh University of Health Sciences (BUHS), Dhaka, Bangladesh

Corresponding author emails: elkadifatimazohra@yahoo.fr, fatimazohra.elkadi@univ-sba.dz

Abstract

Opuntia ficus-indica (OFI) belongs to the Cactaceae family grown in arid regions, including Algeria. Various parts of OFI have been widely used in different countries for their therapeutic properties. Due to the lack of scientific evidence, the consumption of OFI cladodes remains still limited. Therefore, the present study aimed to evaluate the physicochemical and phytochemical properties of OFI cladodes from various harvesting areas in Algeria. Qualitative phytochemicals analysis was measured by following the standard methods, antioxidant activity was measured by the DPPH method, and quantification of secondary metabolites and ferric reducing antioxidant power (FRAP) tests were conducted using spectrophotometric methods respectively. The study revealed that cladodes from Medea station showed a significant yield, however, the content of ash, lipids, and potassium significantly differed from one region to another. While compared to the other stations, the phytochemical study showed that the ethanolic extract of cladodes from the Ain Temouchent station had significant levels of total polyphenols and flavonoids, hydrolyzable tannins and condensed tannins, steroids, alkaloids, and triterpenoids, respectively. Moreover, all of the extracts from different regions contained a significant amount of strong antioxidant activity. It can be concluded that the OFI cladodes contain a significant amount of phytochemicals and strong antioxidant activities, which could lead to new opportunities in various sectors including health, cosmetic, and food sectors.

Key words: *Opuntia ficus indica*, secondary metabolites, antioxidant, phytochemicals, cladodes.

INTRODUCTION

Opuntia ficus-indica (OFI), locally known as prickly pear or nopal cactus, is a member of the Cactaceae family and is grown in arid regions, including Algeria (Aruwa et al., 2018). Since the ancient period due to the high nutrient content (fiber, pectin, mucilage, lignin, cellulose, and hemicellulose) different parts of OFI have been used for various purposes. Traditionally, fruit is consumed by humans, while the cladodes are utilized as animal feed (goats, sheep, and bovines). Additionally, OFI is extensively employed to prevent soil erosion and combat desertification (Slimen et al., 2017). Prickly pear cladodes have been widely consumed as vegetables in various forms and are considered health-promoting foods because

they contain plenty of potentially active nutrients including calcium, fiber as well as phenolic contents (Rodrigues et al., 2023).

Various researchers claimed that the different parts of OFI are considered enormous sources of bioactive compounds such as phenols, flavonoids, anthocyanins, betaxanthin and betacyanin, vitamin C, carotenoids, vitamin E, antioxidants, lignins, sterols, esters, saponins, alkaloids, carbohydrates, amino acids, fatty acids, betalains, as well as sterols (Mendonca et al., 2019; Msaddak et al., 2017)

Moreover, researchers have reported that different parts of OFI possesses substantial therapeutic properties, such as antioxidant, antimicrobial (Smida et al., 2017), antiviral (Rasoulpour et al., 2018), anti-inflammatory (Ammar et al., 2018), anti-proliferative, pro-

apoptotic (Naselli et al., 2014), anti-ulcerogenic (Galati et al., 2003), anti-asthmatic, antidiabetic, anti-rheumatoid (Aragona et al., 2018), and oxidative stress-reducing capabilities (Petruk et al., 2017). Particularly cladodes are commonly used in traditional medicine to treat ulcers, wounds, diabetes, and rheumatic pain. (Msaddak et al., 2017). In addition, cladodes have not only therapeutic activities but are also used in various fields, notably in agro-food as low-calorie marmalades, juices, drinks, jams, and sweeteners (Aruwa et al., 2018), in meat preservation (Palmeri et al., 2018), cookies (Msaddak et al., 2015), as well as in cosmetic industries. Numerous investigations have shown that environmental variables, plant age, post-harvest treatment, and species influence the bioactive characteristics of cladodes. (Figueroa-Pérez et al., 2018; da Cruz Filho et al., 2019). Limited research has been conducted on the physicochemical and phytochemical properties of OFI cladodes found in different regions of Algeria. Therefore, the study aimed to investigate the physicochemical and phytochemical characteristics of OFI cladodes ethanolic extracts derived from various regions in Algeria.

MATERIALS AND METHODS

Bioclimatic conditions and sampling stations

During December 2023, OFI cladodes were collected from three different areas in Algeria, such as Station 1: Tessala (Sidi Bel Abbes); Station 2: Terga (Ain Temouchaint); Station 3: Ksar Boukhari (Medea), in terms of relief and climate, and their bioclimatic conditions are represented in Table 1.

Table 1. Bioclimatic conditions of OFI cladode in different areas in Algeria (S: station)

Parameters	Stations		
	S1	S2	S3
Latitude (UTM)	3526978	35 41 85	35 53 20
Longitude (UTM)	0076408	-1.17753	24456
Altitude (m)	1061	34	621
Temperature (°C)	27.2	19.1	28
Precipitation (mm)	427	407	545
Wind speed (km/h)	21	11.5	16

Three different areas of collected OFI cladodes were washed with tap water and then cut into 2 cm slices. Afterward, the cladodes slices were

dried at 40°C in an oven. The dried samples were ground by using a mortar and pestle and preserved in aluminium-covered glass containers in a dry place for analysis of phytochemicals and physicochemical properties.



Figure 1. *Opuntia ficus-indica* (OFI): (A) Sliced cladode (B) Hand-ground cladode; (C) Cladode powder

Preparations of OFI ethanolic extracts

OFI-preserved cladode powder was mixed with 70% ethanolic solution and left for 24 hours at room temperature (Majhenic et al., 2007). With the help of Whatman filter paper, the solution was filtered and by using a rotary evaporator (RE100-Pro DLAB USA), the solution was concentrated. Afterward, the concentrated solution was placed in an oven (40°C) for complete drying. Additionally, ethanolic (70%) extraction of cladodes collected from different stations was performed using the same procedure. Finally, the extracts were stored at 4°C for physicochemical and phytochemical characteristics studies.

Physicochemical analysis

For the determination of ash, moisture, and titratable acidity content of the OFI cladode extracts, NF V 03-707, 2000, and 1998 protocols were followed. Soxhlet extraction method was used to determine fat content of extracts, (A.O.A.C., 1995). For protein determination, the Bradford et al. (1976) stated method had been used.

The following equation was used to evaluate total carbohydrates:

Total carbohydrates (g/100 g) = 100 – (m fat + m ash + m proteins).

In addition, sodium and potassium captions were quantified using a Corning 400 spectrophotometer through flame spectrophotometry.

Qualitative phytochemical analysis

A qualitative phytochemical analysis of the cladode extracts was performed, using the standard procedures which was described by Trease & Evans (1987).

Steroids and triterpenoids

OFI cladode extract (1 mL) was mixed with chloroform (2 mL) as well as concentrated sulphuric acid (3 mL). Afterward, the red appearance in the inferior layer indicates the presence of steroids while the yellow color in the superior layer indicates the presence of triterpenoids.

Hydrolyzable tannins & condensed tannins

OFI extracts (1 mL) were added with 2 mL of water and a few drops of 1% FeCl₃ solution. The blue-black and blue-green colors indicate the presence of hydrolyzable tannins as well as condensed tannins, respectively.

Phenols

The OFI extracts were diluted with distilled water in a ratio of 1:4, and a few drops of 1% FeCl₃ solution were added. The presence of a dark green or dark blue color indicates the existence of phenols.

Flavonoids

Cladode extracts (1 mL) were added to test tubes along with HCl, and a few fragments of magnesium ribbons were also added. The slight pink color in the test tubes indicated the presence of flavonoids in the extracts.

Anthraquinones

The extracts were dissolved in chloroform as well as 10% KOH. Afterward, red coloration indicates the presence of anthraquinones.

Alkaloids

The extracts were treated with concentrated hydrochloric acid, and a few drops of Mayer's reagent were added. The formation of a precipitate suggests the presence of alkaloids.

Reducing compound

The extracts were mixed with Fehling's liqueur solution and heated in a water bath (70°C) for up to 2-3 min and brick-red precipitation indicates the presence of reducing compounds.

Quantification of phytochemicals

The total polyphenol content was determined by using a standard method (Qusti et al., 2010). Each extract was mixed with 2.5 mL of Folin-Ciocalteu reagent (10%) and 2 mL of Na₂CO₃ solution (7.5%). After incubation (30 minutes), the absorbance was measured at 765 nm

wavelength (756 s visible spectrophotometer, China). Moreover, a standard curve was prepared using a range of gallic acid concentrations (10-100 µg/mL), and the polyphenol content was expressed in milligrams of gallic acid equivalent per gram of dry matter (mg GAE/g DM). Flavonoid content was determined by using the AlCl₃ colorimetric assay method (Chang et al., 2002). Each extract was diluted with distilled water (2 mL) as well as NaNO₂ (5%). The mixture was incubated for 5 minutes. Subsequently, 0.1 mL of AlCl₃ (10%) and 0.1 mL of NaOH (4%) were added, and finally, distilled water was added to bring the total volume to 5 mL. After a 15-minute incubation, the absorbance was measured at 510 nm wavelength (756 s visible spectrophotometer, China) (Kanazawa et al., 2012). In addition, quercetin (10-100 µg/mL) was used as the reference compound for the calibration curve, and the total flavonoid contents were expressed as milligrams of quercetin equivalent per gram of dry matter (mg QE/g DM). Hydrolyzable tannins were determined by following the assay of Mole & Waterman, (1987). The absorbance of the prepared mixture was measured at 660 nm wavelength.

$HT = OD \times (M \times V) / (E_{moles} \times W)$

HT%: percentage of hydrolyzable tannins; OD: optical density; M: 300; V: volume of extract used; E_{moles}: 2169 gallic acid; W: sample weight.

To determine condensed tannins, each extract (0.1-0.5 mL) was mixed with vanillin and methanol (4%). After vigorous shaking, concentrated HCl was added. The absorbance was measured at 500 nm of wavelength after a 20-minute incubation (Costa et al., 2015).

The calibration curve was established using catechin as a standard, and the results were expressed as mg of catechin equivalent per gram of dry matter (mg CE/g DM).

Antioxidant activity

The antioxidant activity was evaluated by using the DPPH method (Benhamou, 2013). Different concentrations of each extract were added to the freshly prepared methanolic DPPH solution (0.1 mM). The negative control consisted of methanol mixed with a methanolic DPPH solution. After incubation in the dark for 30 minutes at room temperature, the absorbance was measured at 515 nm wavelength.

The ferric-reducing antioxidant power (FRAP) activity was conducted by using a spectrophotometric method (Oyaizu et al., 1986). The extracts were mixed with phosphate buffer (0.2 M) (pH: 6.6) and 1% $K_3[Fe(CN)_6]$ solutions. Afterward, to stop the reaction, 10% of TCA was added. After centrifugation, the supernatant was mixed with distilled water and 0.1% of $FeCl_3$ solution. In addition, a standard ascorbic acid (10-100 $\mu\text{g/mL}$) was used, and finally, absorbance was measured at 700 nm wavelength.

Statistical analysis

Data was analyzed with Microsoft Excel 2010. All the data were represented as mean $\pm$ standard deviation ($M \pm SD$), with a significance value of $p < 0.05$. All the test were done three times to avoid errors.

RESULTS AND DISCUSSIONS

Yield values of the OFI extracts

After completely drying, the ethanol extracts from the cladodes of *Opuntia ficus-indica* (OFI) were brown-orange and had a sticky gel-like appearance. Among the three different stations of OFI cladodes, station 3 Ksar Boukhari (Medea) had a significantly higher ($p < 0.01$) extract yield ($28.77 \pm 1.62\%$) value (Figure 2). These variations in extract yield may be attributed to the genetic properties of the plant, geographical origin, harvesting season, and the extraction method employed (Kadda et al., 2022).

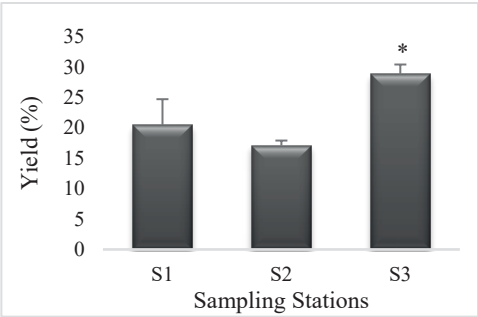


Figure 2. Cladode extraction yield from different harvesting stations. * $p < 0.01$

Chemical composition of OFI cladodes

The various physicochemical parameters (ash, humidity, titrable acidity, lipids, protein, carbohydrate, potassium, and sodium) were measured in collected OFI cladodes from the

different stations of Algeria which are interpreted in Table 2.

The ash content of different OFI cladodes varied from 25 ± 0.001 to 35.01 ± 0.001 g/100 g, while compared to the other stations, station 3 exhibited a significant ($p < 0.05$) value of ash. The mineral content of a plant sample is quantified by the ash value. In Mexico, a recent study confirmed that the ash value of 135-day-old cladodes OFI was 24.30%. (Hernandez et al., 2011). In addition, the Moroccan regions cultivated OFI cladodes ash value was between $25.12 \pm 0.05\%$ and $25.14 \pm 0.05\%$, respectively (Razzak et al., 2024).

The moisture content of the harvested cladodes ranges from 88.67 ± 0.006 to 91.61 ± 0.003 grams/100 grams across all the OFI cladodes extracts. The moisture content of the plant was found to range from 88% to 95%, as reported in studies by Sadok et al. (2014) and Murillo Amador et al. (2002). Another study revealed that the moisture content of OFI cladodes ranged from 90.67% to 94.95% (Razzak et al., 2024). In Mexico, OFI is utilized as a naturally drought-resistant plant due to its high moisture content. The plant also contains a polysaccharide polymer called "mucilage" that can hold large quantities of water, irrespective of the location where it is grown.

The titrable acidity (TA) of cladodes varies between 1 ± 0 and 1.3 ± 0.29 (g CAE/L), is well known that higher acidity values correlate with higher concentrations of tartaric and malic acids (Frîncu et al., 2023).

Table 2. Chemical composition of *Opuntia ficus indica* cladodes (Mean $\pm$ SD; $n = 3$)

Parameters	Sampling stations			ANOVA
	S1	S2	S3	
Ash (g/100 g)	35.01±0.001	31.43±0.001	25±0.001	*
Humidity (g/100 g)	88.67±0.006	90.24±0.018	91.61±0.0033	NS
TA (g CAE/l)	1.3±0.29	1±0	1.17±0.29	NS
Lipids (g/100 g)	9.5±0.86	3.1±0.02	6.1±0.13	NS
Protein (g/100 g)	0.55±0.01	0.51±0.03	0.64±0.05	NS
Carbohydrate (g/100 g)	54.94±0.63	64.96±2.32	68.25±1.08	NS
Potassium (mg/100 g)	125.4±2.25	343.6±1.32	235.4±0.65	*
Sodium (mg/100 g)	2.6±0.35	3.7±0.81	2.1±0.17	NS

S: station; S1: Tessala (Sidi Bel Abbes); S2: Terga (Ain Temouchaint); S3: Ksar Boukhari (Medea); CAE: Citric acid equivalent; TA: titratable acidity; ns: non-significant difference; *:significant difference at $P < 0.05$

The total lipid content of cladodes varies from 3.1 ± 0.02 to 9.5 ± 0.86 g/100 g, which is similar to the findings of El Kossori et al. (1998) ($6.77 \pm 0.51\%$) and Ozcan et al. (2011) ($5.0 \pm 0.9\%$). The total protein contents ranged from 0.51 ± 0.03 to 0.64 ± 0.05 g/100 g, which is consistent with the findings of Stintzing & Carle (2005) (0.1-1%) and Osuna-Martínez et al. (2014) (0.5-1%). The carbohydrate content of the cladodes studied ranges from 54.94 ± 0.63 to 68.25 ± 1.08 g /100 g, which is consistent with the findings of Ramírez-Moreno et al. (2013) from Mexico (60.77%). Cladodes are believed to be high in minerals, particularly calcium, oxalates, and mucilage (Boutakiout et al., 2015). Potassium makes up 60% of the total ash content (166 mg/100 g fresh weight), while sodium is present at 2 mg/100 g fresh weight (de Chávez et al., 1995). The current study found exceptionally high levels of potassium, with a maximum of 343.6 ± 1.32 mg/100 g, and sodium levels ranging between 2.1 ± 0.17 and 3.7 ± 0.81 mg/100. The potassium content of the cladodes at station 2 was significantly ($p < 0.05$) higher compared to the potassium content at the other stations. The variability of findings can be attributed to various factors, such as pH, water availability, texture, geographical location, climate, soil characteristics, growing site, seasons and plant age, cultivation practices, fertilizer application, irrigation, genetic lineage, and agronomic and environmental conditions (Ginestra et al., 2009).

Phytochemicals

Quantitative phytochemical screening of *Opuntia ficus-indica* (OFI) cladodes extracts revealed that there were an abundant source of various bioactive compounds such as polyphenols, tannins, flavonoids, steroids, triterpenoids, and alkaloids, while anthraquinones and reducing compounds were absent (Table 3).

Existing data supports that *Opuntia ficus-indica* (OFI) cladodes are an enormous source of essential phytochemicals, such as phenols, flavonoids, tannins, steroids, saponins, alkaloids, glycosides, phlobatannins, and amino acids (Welegerima et al., 2018) (Dib et al., 2013). Phytochemicals derived from OFI cladodes have demonstrated remarkable

biological activities, including antioxidant properties (Espinosa et al., 2015), antibacterial (López et al., 2015) and antifungal activities (Zabka et al., 2013).

Table 3. Qualitative phytochemical analysis of *Opuntia ficus-indica* cladodes (S: station)

Phytochemicals	Sampling stations		
	S1	S2	S3
Polyphenols	+++	+++	+
Tannins	++	+++	+++
Flavonoids	++	++	+++
Steroids	+	+	+
Triterpenoids	+++	+	+
Alkaloids	++	++	++
Anthraquinones	-	-	-
Reducing compounds	-	-	-
+ (present), (-) absent			

The total phenolic, flavonoids, and tannins contents were evaluated using spectrophotometric techniques, as shown in Figure 3.

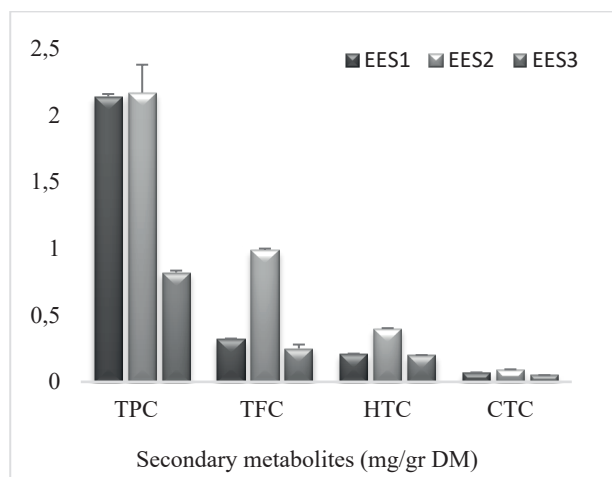


Figure 3. Total phenolic, flavonoids and tannins content of *Opuntia ficus-indica* cladodes

S: station; TPC: Total phenolic content (mg GAE/gr DM); TFC: Total flavonoid content (mg QE /gr DM); HTC: Hydrolysable tannins content (mg GAE/gr DM); CTC: Condensed tannins content (mg CE/gr DM). CE: Catechin equivalent; GAE: Gallic acid equivalent; QE: Quercetin equivalent; DM: dry matter

The total phenolic compounds analysis indicated that the OFI cladodes extract from station 2 in Ain Temouchent contained a significant amount of polyphenols, measuring 2.17 ± 0.21 GAE/g DW. Flavonoids represented the largest class, accounting for 45.62% of the total phenolic content. In contrast, hydrolyzable and condensed tannins

were present in smaller quantities, contributing 18.43% and 4.14% of the total phenolic content, respectively. These values are consistent with those reported in the literature by Jaramillo-Flores et al. (2003), who found 1.589 mg of polyphenols per gram of dry matter, and by Bensadon et al. (2010), who reported polyphenol contents of 2.69 and 3.71 mg/g. Similarly, the total flavonoid content values are in alignment with findings from several other studies (Kuti, 2004; Fernández-López et al., 2010). The variations between the studied extracts may be attributed to differences in the cultivar and genetic factors, growth conditions, harvesting time, ripeness level, fruit processing methods, and, most importantly, the techniques used for determination (De Wit et al., 2020).

Assessment of antioxidant activity

Antioxidant activity is a key mechanism through which fruits and vegetables offer health benefits. The high levels of polyphenols in prickly pear cladodes contribute significantly to their potent antioxidant activity, due to their strong ability to scavenge free radicals and chelate metal ions involved in free radical formation (El-Hawary et al., 2020).

In current study, antioxidant potential of the OFI cladodes extracts was assessed using two in vitro methods: DPPH free radical scavenging and iron reduction assays.

Table 4. IC50 values of DPPH Free-Radical-Scavenging and the iron reduction assays of *Opuntia ficus-indica* cladodes extracts

Samples	DPPH IC50* (mg/mL)	FRAP** (mM Fe ²⁺)
AA	0.024 ±0.007	1.22 ±0.07
EES1	0.91 ± 0.004	0.57 ±0.01
EES2	1.24 ± 0.001	0.76 ±0.008
EES3	1.11 ±0.009	0.48 ±0.04

Values were the means of three replicates ± standard deviation. *IC50 value (mg/mL of extract), inhibitory concentration 50%; DPPH radicals were scavenged by 50%; **Determined at 1 mg/mL

The results indicate that all the extracts possess substantial antioxidant activity and some degree of iron-reducing capacity, although the differences between the extracts are not statistically significant. In addition, it was found that the OFI cladodes extract from station 2 in Ain Temouchent exhibited significant antioxidant activity (1.24 ± 0.001)

and FRAP value (0.76 ± 0.008) compared to the positive control, ascorbic acid (AA). Previous studies supported that OFI cladodes are rich in polyphenol compounds and Vitamin E complexes, which serve as effective radical scavengers, resulting in their strong antioxidant activities (Lanuzza et al., 2017; Kiros et al., 2016).

CONCLUSIONS

The findings of the present study show that *Opuntia ficus-indica* (OFI) cladodes from three different regions in Algeria are a significant source of physicochemical (ash, humidity, titrable acidity, lipids, protein, carbohydrate, potassium, and sodium) and phytochemical compounds (polyphenols, tannins, flavonoids, steroids, triterpenoids, and alkaloids), as well as strong antioxidant activities. Additionally, the location where the cladodes are harvested and specific climatic conditions have an impact on the quality and quantity of phytochemicals present in the cladodes. Therefore, *Opuntia ficus-indica* cladodes can be considered readily available and valuable natural sources of antioxidants and dietary supplements for use in the medical, food, and cosmetic industries.

ACKNOWLEDGEMENTS

The authors express their appreciation and gratitude to all the members of project No: D00L01UN220120230002, under the supervision of Professor El Kadi Fatima Zohra, and funded by the Algerian Ministry of Higher Education and Scientific Research.

REFERENCES

Ammar, I., Salem, M. B., Harrabi, B., Mzid, M., Bardaa, S., Sahnoun, Z., & Ennouri, M. (2018). Anti-inflammatory activity and phenolic composition of prickly pear (*Opuntia ficus-indica*) flowers. *Industrial Crops and Products*, 112, 313-319.

Aragona, M., Lauriano, E. R., Pergolizzi, S., & Faggio, C. J. N. P. R. (2018). *Opuntia ficus-indica* (L.) Miller as a source of bioactivity compounds for health and nutrition. *Natural product research*, 32(17), 2037-2049.

Aruwa, C. E., Amoo, S. O., & Kudanga, T. (2018). *Opuntia* (Cactaceae) plant compounds, biological activities and prospects - A comprehensive review. *Food Research International*, 112, 328-344.

- Benhammou, N., Bekkara, F. A., & Panovska, T. K. (2009). Antioxidant activity of methanolic extracts and some bioactive compounds of *Atriplex halimus*. *Comptes Rendus. Chimie*, 12(12), 1259-1266.
- Bensadón, S., Hervert-Hernández, D., Sáyago-Ayerdi, S. G., & Goñi, I. (2010). By-products of *Opuntia ficus-indica* as a source of antioxidant dietary fiber. *Plant foods for human nutrition*, 65, 210-216.
- Boutakiout, A., Elothmani, D., Mahrouz, M., & Hanine, H. (2015). Effect of season on proximate composition of cladode juice of two species of Cactaceae. *International Journal of Technology Enhancements and Emerging Engineering Research, New Delhi*, 3(1), 3.
- Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical biochemistry*, 72(1-2), 248-254.
- Costa, R. M., Vaz, A. F., Xavier, H. S., Correia, M. T., & Carneiro-da-Cunha, M. G. (2015). Phytochemical screening of *Phthirusa pyrifolia* leaf extracts: Free-radical scavenging activities and environmental toxicity. *South African Journal of Botany*, 99, 132-137.
- Chang, C. C., Yang, M. H., Wen, H. M., & Chern, J. C. (2002). Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *Journal of food and drug analysis*, 10(3).
- da Cruz Filho, I. J., da Silva Barros, B. R., de Souza Aguiar, L. M., Navarro, C. D. C., Ruas, J. S., de Lorena, V. M. B., ... & Maior, A. M. S. (2019). Lignins isolated from Prickly pear cladodes of the species *Opuntia ficus-indica* (Linnaeus) Miller and *Opuntia cochenillifera* (Linnaeus) Miller induces mice splenocytes activation, proliferation and cytokines production. *International journal of biological macromolecules*, 123, 1331-1339.
- de Chávez, M. M., Chávez, A., Valles, V., & Roldán, J. A. (1995). The nopal: a plant of manifold qualities. *Plants in Human Nutrition*, 77, 109-134.
- De Wit, M., Du Toit, A., Osthoff, G., & Hugo, A. (2020). Antioxidant content, capacity and retention in fresh and processed cactus pear (*Opuntia ficus-indica* and *O. robusta*) fruit peels from different fruit-colored cultivars. *Frontiers in Sustainable Food Systems*, 4, 133.
- Dib, H., Beghdad, M. C., & Belarbi, M. (2013). Phytochemical study of Algerian *Opuntia ficus-indica*. *Belarbi Annals of Biological Research*, 4(2), 185-189.
- 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.
- El-Hawary, S. S., Sobeh, M., Badr, W. K., Abdelfattah, M. A., Ali, Z. Y., El-Tantawy, M. E., ... & Wink, M. (2020). HPLC-PDA-MS/MS profiling of secondary metabolites from *Opuntia ficus-indica* cladode, peel and fruit pulp extracts and their antioxidant, neuroprotective effect in rats with aluminum chloride induced neurotoxicity. *Saudi Journal of Biological Sciences*, 27(10), 2829-2838.
- Espinosa, R. R., Inchingolo, R., Alencar, S. M., Rodriguez-Estrada, M. T., & Castro, I. A. (2015). Antioxidant activity of phenolic compounds added to a functional emulsion containing omega-3 fatty acids and plant sterol esters. *Food chemistry*, 182, 95-104.
- Fernández-López, J. A., Almela, L., Obón, J. M., & Castellar, R. (2010). Determination of antioxidant constituents in cactus pear fruits. *Plant Foods for Human Nutrition*, 65, 253-259.
- Figueroa-Pérez, M. G., Pérez-Ramírez, I. F., Paredes-López, O., Mondragón-Jacobo, C., & Reynoso-Camacho, R. (2018). Phytochemical composition and in vitro analysis of nopal (*O. ficus-indica*) cladodes at different stages of maturity. *International Journal of Food Properties*, 21(1), 1728-1742.
- Galati, E. M., Mondello, M. R., Giuffrida, D., Dugo, G., Miceli, N., Pergolizzi, S., & Taviano, M. F. (2003). Chemical characterization and biological effects of Sicilian *Opuntia ficus indica* (L.) Mill. fruit juice: antioxidant and antiulcerogenic activity. *Journal of agricultural and food chemistry*, 51(17), 4903-4908.
- Ginestra, G., Parker, M. L., Bennett, R. N., Robertson, J., Mandalari, G., Narbad, A., ... & Waldron, K. W. (2009). Anatomical, chemical, and biochemical characterization of cladodes from prickly pear [*Opuntia ficus-indica* (L.) Mill.]. *Journal of agricultural and food chemistry*, 57(21), 10323-10330.
- Haile, K., Mehari, B., Atlabachew, M., & Chandravanshi, B. S. (2016). Phenolic composition and antioxidant activities of cladodes of the two varieties of cactus pear (*Opuntia ficus-indica*) grown in Ethiopia. *Bulletin of the Chemical Society of Ethiopia*, 30(3), 347-356.
- Hernández-Urbiola, M. I., Pérez-Torrero, E., & Rodríguez-García, M. E. (2011). Chemical analysis of nutritional content of prickly pads (*Opuntia ficus indica*) at varied ages in an organic harvest. *International journal of environmental research and public health*, 8(5), 1287-1295.
- Jaramillo-Flores, M. E., González-Cruz, L., Cornejo-Mazon, M., Dorantes-Alvarez, L., Gutierrez-Lopez, G. F., & Hernandez-Sanchez, H. (2003). Effect of thermal treatment on the antioxidant activity and content of carotenoids and phenolic compounds of cactus pear cladodes (*Opuntia ficus-indica*). *Food science and technology international*, 9(4), 271-278.
- Julkunen-Tiitto, R. (1985). Phenolic constituents in the leaves of northern willows: methods for the analysis of certain phenolics. *Journal of agricultural and food chemistry*, 33(2), 213-217.
- Kanazawa, K., Hashimoto, T., Yoshida, S., Sungwon, P., & Fukuda, S. (2012). Short photoirradiation induces flavonoid synthesis and increases its production in postharvest vegetables. *Journal of agricultural and food chemistry*, 60(17), 4359-4368.
- Kadda, S., Belabed, A., Loukili, E. H., Hammouti, B., & Fadlaoui, S. (2022). 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. *Environmental Science and Pollution Research*, 1-9.
- Kuti, J. O. (2004). Antioxidant compounds from four *Opuntia* cactus pear fruit varieties. *Food chemistry*, 85(4), 527-533.
- Lanuzza, F., Occhiuto, F., Monforte, M. T., Tripodo, M. M., D'Angelo, V., & Galati, E. M. (2017). Antioxidant phytochemicals of *Opuntia ficus-indica* (L.) Mill. cladodes with potential anti-spasmodic activity. *Pharmacognosy magazine*, 13(Suppl 3), S424.
- López, C. C., Mazzarrino, G., Rodríguez, A., Fernández-López, J., Pérez-Álvarez, J. A., & Viuda-Martos, M. (2015). Assessment of antioxidant and antibacterial potential of borojo fruit (*Borojoa patinoi* Cuatrecasas) from the rainforests of South America. *Industrial Crops and Products*, 63, 79-86.
- Majhenič, L., Škerget, M., & Knez, Ž. (2007). Antioxidant and antimicrobial activity of guarana seed extracts. *Food chemistry*, 104(3), 1258-1268.
- Mendonça, R. D., Carvalho, N. C., Martin-Moreno, J. M., Pimenta, A. M., Lopes, A. C. S., Gea, A., ... & Bes-Rastrollo, M. (2019). Total polyphenol intake, polyphenol subtypes and incidence of cardiovascular disease: The SUN cohort study. *Nutrition, Metabolism and Cardiovascular Diseases*, 29(1), 69-78.
- Frincu, M., Dumitrache, C., Petre, A. C., Mot, A., Teodorescu, R. I., Bărbulescu, D. J., Tudor, V., & Matel, F. (2023). Physico-chemical characterization of some sources of grape marc from Pietroasa vineyard. *AgroLife Scientific Journal*, 12(1), 81-86.
- Mole, S., & Waterman, P. G. (1987). A critical analysis of techniques for measuring tannins in ecological studies: I. Techniques for chemically defining tannins. *Oecologia*, 72, 137-147.
- Msaddak, L., Siala, R., Fakhfakh, N., Ayadi, M. A., Nasri, M., & Zouari, N. (2015). Cladodes from prickly pear as a functional ingredient: Effect on fat retention, oxidative stability, nutritional and sensory properties of cookies. *International Journal of Food Sciences and Nutrition*, 66(8), 851-857.
- Msaddak, L., Abdelhedi, O., Kridene, A., Rateb, M., Belbahri, L., Ammar, E., ... & Zouari, N. (2017). *Opuntia ficus-indica* cladodes as a functional ingredient: bioactive compounds profile and their effect on antioxidant quality of bread. *Lipids in health and disease*, 16, 1-8.
- Murillo Amador, B., Diéguez, T., Garibay, N., & García, A. (2002). El nopal cultivo forrajero sostenible para el noroeste de México.
- Naselli, F., Tesoriere, L., Caradonna, F., Bellavia, D., Attanzio, A., Gentile, C., & Livrea, M. A. (2014). Anti-proliferative and pro-apoptotic activity of whole extract and isolated indicaxanthin from *Opuntia ficus-indica* associated with re-activation of the onco-suppressor p16INK4a gene in human colorectal carcinoma (Caco-2) cells. *Biochemical and Biophysical Research Communications*, 450(1), 652-658.
- Osuna-Martínez, U., Reyes-Esparza, J., & Rodríguez-Fragoso, L. (2014). Cactus (*Opuntia ficus-indica*): A Review on its Antioxidants Properties and Potential Pharmacological Use in Chronic Diseases. *Nat Prod Chem Res* 2: 153. *Opuntia ficus-indica*.
- Oyaizu, M. (1986). Studies on products of browning reaction antioxidative activities of products of browning reaction prepared from glucosamine. *The Japanese journal of nutrition and dietetics*, 44(6), 307-315.
- Özcan, M. M., & Al Juhaimi, F. Y. (2011). Nutritive value and chemical composition of prickly pear seeds (*Opuntia ficus-indica* L.) growing in Turkey. *International journal of food sciences and nutrition*, 62(5), 533-536.
- Palmeri, R., Parafati, L., Restuccia, C., & Fallico, B. (2018). Application of prickly pear fruit extract to improve domestic shelf life, quality and microbial safety of sliced beef. *Food and Chemical Toxicology*, 118, 355-360.
- Petruck, G., Di Lorenzo, F., Imbimbo, P., Silipo, A., Bonina, A., Rizza, L., ... & Lanzetta, R. (2017). Protective effect of *Opuntia ficus-indica* L. cladodes against UVA-induced oxidative stress in normal human keratinocytes. *Bioorganic & Medicinal Chemistry Letters*, 27(24), 5485-5489.
- Qusti, S. Y., Abo-khatwa, A. N., Lahwa, M. B., & Qusti, S. (2010). Screening of antioxidant activity and phenolic content of selected food items cited in the Holly Quran. *EJBS*, 2(1), 40-51.
- Ramírez-Moreno, E., Córdoba-Díaz, D., de Cortes Sánchez-Mata, M., Díez-Marqués, C., & Goñi, I. (2013). Effect of boiling on nutritional, antioxidant and physicochemical characteristics in cladodes (*Opuntia ficus-indica*). *LWT-Food Science and Technology*, 51(1), 296-302.
- Rasoulpour, R., Afsharifar, A., & Izadpanah, K. (2018). Antiviral activity of prickly pear (*Opuntia ficus-indica* (L.) Miller) extract: Opuntin B, a second antiviral protein. *Crop protection*, 112, 1-9.
- Razzak, S., Aouji, M., Zirari, M., Benchehida, H., Taibi, M., Bengueddour, R., ... & Taboz, Y. (2024). Nutritional composition, functional and chemical characterization of Moroccan *Opuntia ficus-indica* cladode powder. *International Journal of Food Properties*, 27(1), 1167-1179.
- Rodrigues, C., Paula, C. D. D., Lahbouki, S., Meddich, A., Outzourhit, A., Rashad, M., ... & Souza, V. G. (2023). *Opuntia* spp.: an overview of the bioactive profile and food applications of this versatile crop adapted to arid lands. *Foods*, 12(7), 1465.
- Sadok, H. T., Aid, F., Doumandji, A., & Bellal, M. (2014). Effet du jus de cladodes d'*Opuntia ficus indica* sur la fermentation du lait et la croissance des bactéries lactiques et probiotiques. *Nature & Technology*, 11, 17.
- Slimen, I. B., Najar, T., & Abderrabba, M. (2016). *Opuntia ficus-indica* as a source of bioactive and nutritional phytochemicals. *Journal of Food and Nutrition Sciences*, 4(6), 162-169.
- Smida, A., Ncibi, S., Taleb, J., Saad, A. B., Ncib, S., & Zourgui, L. (2017). Immunoprotective activity and antioxidant properties of cactus (*Opuntia ficus-indica*) extract against chlorpyrifos toxicity in rats. *Biomedicine & Pharmacotherapy*, 88, 844-851.

- Stintzing, F. C., & Carle, R. (2005). Cactus stems (*Opuntia* spp.): A review on their chemistry, technology, and uses. *Molecular nutrition & food research*, 49(2), 175-194.
- Trease, G. E., & Evans, W. C. (1978). Pharmacology, 11th. *Bailliere Tindall Ltd., London*, 60-75.
- Welegerima, G., Zemene, A., & Tilahun, Y. (2018). Phytochemical composition and antibacterial activity of *Opuntia ficus-indica* cladodes extracts. *J. Med. Plants Stud.*, 6, 243-246.
- Zabka, M., & Pavela, R. (2013). Antifungal efficacy of some natural phenolic compounds against significant pathogenic and toxinogenic filamentous fungi. *Chemosphere*, 93(6), 1051-1056.