Print ISSN: 0375-9237
Online ISSN: 2357-0350

EGYPTIAN JOURNAL OF BOTANY (EJBO)

Phytochemicals, antioxidant properties and anti-inflammatory capacity of hydro-soluble seed extract of *Opuntia ficus-indica L.*

Touati N., Sakhraoui. A, Hihat S., Blando. F.

PUBLISHED BY
THE EGYPTIAN
BOTANICAL SOCIETY

Phytochemicals, antioxidant properties and anti-inflammatory capacity of hydro-soluble seed extract of *Opuntia ficus-indica L.*

Touati N.[1,2], Sakhraoui. A[3], Hihat S.[2,4], Blando. F.[5]

[1]*Laboratory of Health and Environment, University Mohamed El Bachir El Ibrahimi of Bordj Bou Arreridj, 34030 Bordj Bou Arreridj, Algeria*
[2]*Department of Food Sciences, Faculty of life and Natural Sciences and Earth and Universe, University Mohamed El Bachir El Ibrahimi of Bordj Bou Arreridj, 34030 Bordj Bou Arreridj, Algeria*
[3]*Laboratory of Characterization and Valorization of Natural Resources, University Mohamed El Bachir El Ibrahimi of Bordj Bou Arreridj, 34030 Bordj Bou Arreridj, Algeria*
[4]*Laboratory of Biomathematics Biophysic, Biochemical and Scientometry, University of Bejaia, 06000 Bejaia, Algeria*
[5]*Institute of Sciences of Food Production (ISPA), National Research Council (CNR), Research Unit of Lecce, 73100 Lecce, Italy*

This study aimed to highlight the importance of the hydro-soluble *Opuntia ficus-indica* L. seeds extract. To this end, various *in vitro* tests were performed to determine the levels of antioxidants (total polyphenols (TP), total flavonoids (TF), and condensed tannins (CT)) and to evaluate the antioxidant capacity (radical Diphenyl 1-Picrylhydrazyl scavenging activity (DPPH), ferric reducing antioxidant power (FRAP), Hydrogen peroxide scavenging activity (H_2O_2), and β-carotene bleaching scavenging activity (BB)) and anti-inflammatory activity. The results showed that *O. ficus-indica* seeds extract was rich in antioxidant compounds (TP, 905.71±42.50 mg GAE/100 g DW; TF, 50.77±0.08 mg QE/100 g DW; and CT, 98.99±8.19 mg CE/100 g DW). In addition, the seed extract exhibited high antioxidant capacity (DPPH, 248.40±1.06 mg GAE/100 g DW; FRAP, 382.56±1.70 mg GAE/100 g DW; H_2O_2, 62.01±0.57%; and BB, 83.87±1.76%) and high anti-inflammatory capacity. This study provides important insights into the presence of bioactive compounds and antioxidant capacity of hydro-soluble seed extract of *O. ficus-indica*.

Keywords: *O ficus-indica* L., antioxidant substances, antioxidant activity, hydro-soluble extract

ARTICLE HISTORY
Submitted: May 19, 2024
Accepted: November 30, 2024

CORRESPONDENCE TO
Touati N,
Laboratory of Health and Environment, University Mohamed El Bachir El Ibrahimi of Bordj Bou Arreridj, 34030 Bordj Bou Arreridj, Algeria
Email: n.touati@univ-bba.dz
DOI: 10.21608/ejbo.2024.286760.2829

EDITED BY: M. Madany

INTRODUCTION

As the global's demand for natural antioxidants and anti-inflammatory agents continues to rise, understanding the untapped potential of by-products from agricultural processes is becoming increasingly important. Antioxidants are promising agents to protect cellular organelles from free radical damage. Phytochemicals, especially phenolic compounds, are the most important bioactive compounds known for their health benefits (Sakihama et al., 2002; Sakhraoui et al., 2023; Hihat et al., 2024).

O ficus-indica L., is a member of the cactus family (*Cactaceae*), which includes around 130 genera and two thousand species (Ramadan. 2021). It is an emerging species with interest not only for crop production but also as a source of nutraceutical compounds (Blando et al., 2022). The composition of the fruit is highly versatile, making it a significant source of phytochemicals with confirmed biological activities and high value for the food and nutraceutical industries (Barba et al., 2017; Mena et al., 2018; Hihat et al., 2024). *O. ficus-indica* fruits are abundant in ascorbic acid, betalains (such as betanin and indicaxanthin), phenolic acids (including piscidic acid and hydroxybenzoic acid derivatives), flavonoids (such as isorhamnetin, kaempferol, and quercetin glycosides), and carotenoids (mainly lutein) (Cano et al., 2017; García-Cayuela et al., 2019; Gómez-Maqueo et al., 2019). They have considerable nutritional value and multiple biological effects, including antioxidant, anti-inflammatory and anti-diabetic properties (Jung-Woo Kang et al., 2016).

Traditionally, the focus of prickly pear seed processing has been on extracting oil (Ramírez-Moreno et al., 2017), leaving the hydro-soluble fraction, disregarded, and considered as waste. However, this study explores the untapped potential of the water-soluble extract obtained from prickly pear seeds, challenging the common belief that it lacks significant nutritional value. This study provides important insights into utilizing the full potential of this agricultural by-product, not only in reducing waste but also in meeting the growing demand for natural, health-promoting compounds.

The objective of this study was to highlight the importance of seed extract *O. ficus-indica* by conducting various *in vitro* tests to determine its antioxidant content and evaluate its antioxidant capacity and anti-inflammatory properties.

MATERIALS AND METHODS

Chemicals

DPPH reagents were purchased from Sigma Chemical (Darmstadt, Germany), and Folin–Ciocalteu phenol reagent was obtained from Biochem, Chemopharma

(Montreal, Quebec). All chemicals and solvents used were of analytical grade.

Plant materials

The fruits, which are characterized by yellow-orange coloration indicating ripeness, were harvested in Ain Hamra, in the Bordj Bou Arreridj department (36°15'44'81"S, 4°79'13'85"W) towards the end of August 2020. The thorns were removed from the fruit, which was then washed and peeled. The seeds, separated from the pulp, were ground to a fine powder (< 250 µm) using an electric grinder (SEB 500W) and stored in an airtight box until use.

Microwave-assisted extraction process

A domestic microwave oven (CMW-A2602, Condor, Algeria) with a digital control system for irradiation time and microwave power was used for the extraction. The oven was modified so that the vapors generated during extraction were condensed in the sample. An aliquot of 0.5 g of the powdered sample was extracted with 60% acetone concentration, 900 watts of microwave power and 210 s of irradiation time. The extract was centrifuged at 3000 rpm (SIGMA 3-30ks) for 15 min and the supernatant was stored at 4 °C until further use.

Determination of antioxidant substances

Total polyphenols: Total phenolic compounds content was determined using the Folin-Ciocalteu reagent (Adesegun et al., 2007). An aliquot of 100 µL of the extract was mixed with 800 µL of Folin-Ciocalteu reagent (10%) and 400 µL of sodium carbonate (7%). After 30 min of incubation at room temperature, absorbance was measured at 760 nm against to the blank (made as reported for the sample but with 100 µL of sample solvent). The results were expressed as mg gallic acid equivalent (GAE) per 100 g DW.

Total flavonoids: Total flavonoids content was determined using a colorimetric method (Ayoola et al., 2008). A volume of 2 mL of the extract was mixed with 2 mL of aluminum trichloride reagent (2% in pure methanol). After 10 min of incubation at room temperature, the absorbance was recorded at 420 nm against the blank (made as reported for the sample but with 2 mL of sample solvent). The results were expressed as mg quercetin equivalent (QE) per 100 g DW.

Condensed tannins: Condensed tannins content was determined using the butanol/HCl mixing method (Porter et al., 1986). An aliquot of 400 µL of the extract was mixed with 2 mL of ferrous sulphate acid solution (7.7 mg ferrous ammonium sulphate: $Fe_2(SO_4)_3$ dissolved in 50 mL n-butanol; HCl 3:2 (*v/v*)). After incubation at 95 °C for 15 min, the absorbance was measured at 530 nm against the blank (made as reported for the sample but with 400 µL of sample solvent). The results were expressed as mg cyanidin-3-glucoside equivalent (CE) per 100 g DW.

Evaluation of antioxidant capacity

DPPH radical scavenging activity: The DPPH radical scavenging activity was evaluated using the method outlined by Brand-Williams et al. (1995). A volume of 200 µL of the sample was mixed with 1 mL of a methanolic DPPH solution (60 µM). After incubating for 30 min at room temperature in the dark, the absorbance was read at 517 nm against a blank (prepared similarly but with 200 µL of sample solvent). The results were expressed in mg gallic acid equivalent (GAE) per 100 g DW.

Ferric reducing antioxidant power: The ferric reducing antioxidant power was evaluated according to the method described by Oyaizu (1986). A volume of 2.5 mL of the aqueous extract sample was mixed with 2.5 mL of phosphate buffer (0.2 M; pH 6.6) and 2.5 ml of 1% potassium ferricyanide. After 20 min of incubation at 50 °C, 2.5 mL of 10% trichloroacetic acid solution was added. Then, 2.5 mL of the reaction mixture was diluted with distilled water (v/v) and 500 µl of (0.1%) ferric chloride solution was added. The absorbance was measured at 700 nm against the blank (made as reported for the sample but with 2.5 mL of sample solvent) and the results were expressed as mg gallic acid equivalent (GAE) per 100 g DW.

Hydrogen peroxide scavenging activity: The hydrogen peroxide scavenging activity was measured following the method of Ruch et al. (1989). In hemolysis tubes, 50 µL of the extract was combined with 1 mL of hydrogen peroxide (40 mM in phosphate buffer solution (0.1 mM, pH 7.4)) and 1450 µL of phosphate buffer solution (0.1 mM, pH 7.4). The mixture was incubated for 10 min, and the absorbance was recorded at 230 nm. The control was prepared in the same manner, but the extract was replaced with 60% (*v/v*) acetone. The hydrogen peroxide scavenging activity was then calculated using the given equation (Eq 1).

$$\text{Hydrogen peroxide scavenging activity activi} = \left(\frac{\text{Ac} - \text{At}}{\text{Ac}}\right) \text{x} 100 \quad (1)$$

Where Ac is the absorbance of the control, At is the absorbance of the sample.

***β*-carotene bleaching scavenging activity:** *β*-carotene bleaching scavenging activity was performed according to the method described by Kartal et al. (2007). A β-carotene/linoleic acid emulsion was prepared by dissolving 2 mg of β-carotene in 1 mL of chloroform, followed by the addition of 25 µL of linoleic acid and 200 mg of Tween 40. The chloroform was completely evaporated using rotary evaporation (at 40 °C for 1h) and 100 mL of saturated oxygen distilled water was added. The resulting emulsion was stirred vigorously. A volume of 2.5 mL of the emulsion was mixed with 350 µL of the extract. The test tubes were incubated in the dark at room temperature. Two control tubes were also prepared using the same procedure, one with a reference antioxidant BHT (2 mg/mL) dissolved in methanol (positive control) and the other without antioxidant (negative control), in which the sample was replaced with 350 µL of acetone (60%). The kinetics of emulsion discoloration in the presence and absence of the antioxidant was monitored for 48 h at regular intervals at 490 nm. The relative antioxidant activity (RAA) of the extracts was calculated using the equation Eq (2).

$$\mathrm{RAA\%} = \frac{\mathrm{Abs_{48h}(sample)}}{\mathrm{Abs_{48h}(BHT)}} \times 100 \qquad (2)$$

Where RAA is the relative antioxidant activity, Abs_{48h} (sample) and Abs_{48h} (BHT) were the absorbance of the sample and positive control after 48 hours, respectively.

The anti-inflammatory activity: The anti-inflammatory activity was determined *in vitro* by studying the heat-induced denaturation of bovine serum albumin solution (Kandikattu et al., 2013). Different volumes of the extract were mixed with 1 mL of bovine serum albumin solution (0.2%) prepared in Tris-HCl buffer (50 mM, pH 6.6). The tubes were heated to 37 °C for 15 min and then to 72 °C for 5 min. After cooling the tubes to room temperature, the absorbance was measured at 660 nm. The sodium diclofenac (VOLTARENE®) was used as a standard. The protective effect of the samples against bovine serum albumin solution denaturation was expressed as a percentage inhibition calculated according to the equation Eq (3).

$$\mathrm{I\%} = \frac{\mathrm{Ac-As}}{\mathrm{Ac}} \times 100 \qquad (3)$$

Where I is the inhibition percentage, AS the absorbance of the test sample, and AC is the absorbance of control.

Statistical analysis

The results (n=3) were subjected to a two-factor analysis of variance. Mean values were compared using Fisher's test ($p < 0.05$). All statistical analyses are conducted using Infostat®.

RESULTS AND DISCUSSION

Antioxidant determination

The results of the antioxidants determination including total polyphenols, total flavonoids, and condensed tannins were summarized in Table 1.

Based on the absorbance values of the extract reacted with the Folin-Ciocalteu reagent shown in Table 1, the total polyphenols content was determined to be 905.71±0.50 mg GAE/100 g DW. This value was higher than that reported by Cardador-Martinez et al. (2011) for the same species in cultivars of Mexican origin, which ranged from 337 to 460 mg GAE/100 g. This difference could be attributed to variations in the type of solvent used or differences in storage conditions. The solubility of phenolic compounds can be influenced by factors such as the polarity of the solvent, the polymerization degree of the polyphenols, as well as interactions with other plant compounds leading to the formation of insoluble complexes. Comparable results were recorded by Chaalal et al. (2013) for the ground seed of *O ficus-indica* L, with different values for red and yellow species (298.29 and 316.46 mg GAE/100 g, respectively). This variability in total polyphenols content could indeed be attributed to the ripeness degree of the fruits.

Regarding the total flavonoids content in seed extract *of O ficus-indica* L., the result revealed a value of 50.77±0.08 mg QE/100 g DW (Table 1). Our findings demonstrate a higher flavonoids content compared to that reported by Cardador-Martinez et al. (2011) and Khatabi et al. (2016) on prickly pear seeds, which reported values ranging from 46 to 50 mg QE/100 g. In the case of whole fruits of *O ficus-indica* L., the total flavonoids content was found to be 17.81±0.10 and 15.03±1.36 mg ECa/kg for red and yellow fruits, respectively. Variations in total flavonoids content among studies could be attributed to factors such as fruits color or ripeness. Additionally, it should be noted that total flavonoids content is often expressed using different equivalent standards (quercetin, rutin, catechin), and the choice of standards used can influence the result.

Concerning the determination of condensed tannins content in seed extract of *O ficus-indica* L. was

conducted using a colorimetric method involving the oxidative cleavage of proanthocyanidins with ferrous sulfate (Vermerris and Nicholson, 2007). The results depicted in Table 1 revealed a concentration of 98.99 ± 8.19 mg/100 g DW for condensed tannins in the sample. This value falls below the range reported by Cardador-Martinez et al. (2011), which spanned between 137 and 205 mg ECa/100 g DW. Comparable trends have been observed in the condensed tannins content of seeds from other species, such as seed oranges, where levels varied based on fruit characteristics. Additionally, physico-chemical parameters, including heat and conductivity, could influence the degree of condensed tannins present in the samples (salas et al., 2024). Overall, the antioxidant analyses suggest that seed extract of *O ficus-indica* L. is abundant in bioactive compounds like phenolic compounds, flavonoids, and condensed tannins, which collectively contributed to its antioxidant properties. These results underscore the potential health advantages of hydro soluble seed extract of prickly pear and its promising applications in the formulation of functional foods or nutraceutical products. (Khatabi et al., 2016; Barba et al., 2017).

Antioxidant activities evaluation

The antioxidant capacity of hydro-soluble seed extract of *O ficus-indica* L. was evaluated using four different tests: DPPH radical scavenging activity (DPPH), Ferric reducing antioxidant power (FRAP), Hydrogen peroxide scavenging activity (H_2O_2), and *β*-carotene bleaching scavenging activity (BB). The results are summarized in Table 2.

The free radical DPPH assay is a widely employed method to assess antioxidant activity, involving the reduction of a stable, purple-colored DPPH radical by natural antioxidants or reducing compounds, leading to a color shift to pale yellow (Azman et al., 2019). The outcome of the DPPH radical scavenging capacity test conducted on *O ficus-indica* L. seed extract yielded a value of 248.40±15.06 mg GAE/100 g DW (Table 2). Chaalal et al. (2013) reported ranging from 89.138±6.75 to 114.699±6.78 mg AAE/g DW for Algerian cultivars. Yolmeh et al. (2014), who investigated the entire fruit of *O ficus-indica* L., documented a higher value (547.8 mg GAE/100 g). The disparity in results can be attributed to the intricate and varied chemical composition among distinct species, which collectively contribute to the total antioxidant capacity (Zaghad et al., 2019).

The ferric reducing antioxidant power assay serves as a method to measure the antioxidant capacity of samples by assessing their ability to donate electrons and convert ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}) (Al Juhaimi et al., 2020). Upon evaluating the reducing power of *O ficus-indica* L. seed extract, a concentration of 382.56 ± 7.70 mg GAE/100 g DW was recorded (Table 2). This finding surpasses the values documented by Chaalal et al. (2013) for ground prickly pear seed, which ranged from 1861.55 ± 17.60 to 1978.16 ± 13.18 µg AAE/g across different varieties and falls within the range of results reported by Chougui et al. (2013) for prickly pear seed (32.3 and 51.3 mg AAE/100 g). The presence of hydroxyl groups in phenolic compounds underlies the observed reducing power of the extracts, as these groups can function as electron donors. Reducing power is considered a significant indicator of potential antioxidant activity (Han et al., 2024; Al Juhaimi *et al*., 2020).

The hydrogen peroxide scavenging activity test is utilized to assess a sample's capacity to neutralize hydrogen peroxide, a key player in oxidative stress (Al Juhaimi et al., 2020). The findings outlined in Table 2 reveal an average hydrogen peroxide scavenging activity of 62.01±2.57% for the prickly pear seed extract under investigation. This result falls below the figures reported by Chaalal et al. (2013) for crushed prickly pear seed, ranging from 91.87±1.25 to 93.55±1.32% across different varieties. Similarly, Chougui et al. (2013) documented higher percentages of hydrogen peroxide scavenging (ranging from 76 to 96%). The presence of phenolic compounds in the extract serves as electron donors, facilitating the conversion of hydrogen peroxide into water (Dib et al., 2013). The scavenging capacity against hydrogen peroxide correlates with the phenolic content, underscoring their role in the antioxidant activity.

β-carotene bleaching scavenging activity was employed to assess, *in vitro*, the potential of a substance to hinder lipid peroxidation (Tepe et al., 2005). During this test, linoleic acid undergoes oxidation, generating free radicals that subsequently oxidize *β*-carotene, causing its orange color to fade. However, the presence of an antioxidant can counteract the free radicals produced by linoleic acid, thereby preventing the oxidation, and bleaching of *β*-carotene (Kulisic et al., 2004; Sarikurkcu et al., 2008). The outcome of the *β*-carotene bleaching test (Table 2) reveals a relative antioxidant activity of 83.87±1.76%. The substantial inhibition observed in this study is due to the polarity and chemical composition of the seed extract. An extract that demonstrates inhibition of *β*-carotene bleaching is

Table 1. Contents of total phenolic compounds, flavonoids, and condensed tannins in *O ficus-indica* L.

Antioxidant compounds	Content
Total polyphenols (mg GAE/100g DW)	905.71±0.50
Total flavonoïds (mg QE/100g DW)	50.77±0.08
Condensed tannins (mg CE/100g DW)	98.99±0.19

Table 2. Values of antioxidant activities of *O ficus-indica* L. seed extract.

Antioxidant activities	Values
DPPH radical scavenging activity (mg GAE/100g DW)	248.40±1.06
Ferric reducing antioxidant power (mg GAE/100g DW)	382.56±1.70
Hydrogen peroxide scavenging activity (%)	62.01±0.57
β-carotene bleaching scavenging activity (%)	83.87±1.76

considered a scavenger of free radicals and a primary antioxidant (Hamid et al., 2024). Combining the linoleic acid inhibition assay with the *β*-carotene test is recognized by several authors as a mimetic model for lipid peroxidation in biological membranes (Almeida et al., 2024)**.**

Anti-inflammatory activity

The presence of flavonoids in various parts of *O ficus-indica* L. contributes to its anti-inflammatory capacity by inhibiting important regulatory enzymes. Certain flavonoids have been identified as potent inhibitors of prostaglandin production, which are highly active pro-inflammatory molecules (Murad et al*.,* 2023). To evaluate the anti-inflammatory capacity of the studied prickly pear seed extract, the bovine serum albumin (BSA) protein denaturation method was employed. The protective effect of the seed extracts against thermal denaturation of BSA was expressed as a percentage of inhibition Figure 1, and the inhibition rate was found to be dose dependent.

According to Figure 1 the acetonique extracts of the seed, at different concentrations (50, 100, and 250 mg/mL), exhibited a significant protective effect against the denaturation of BSA induced by heating at 72 °C. The inhibition percentages were 34±0.002, 65 ± 0.003, and 86±0.42%, respectively. These values were significantly higher ($p < 0.05$) than those of diclofenac, a non-steroidal anti-inflammatory drug, which showed inhibition percentages of 23±0.01, 53±0.11, and 60±0.007% at the corresponding concentrations. , these results fall within the findings of Youssef *et al*. (2021) who found that the *opuntia* genus has an important anti-inflammatory effect.

Previous studies have reported that certain non-steroidal anti-inflammatory drugs, including diclofenac, salicylic acid, phenylbutazone, and indomethacin, not only inhibit the synthesis of pro-inflammatory prostaglandins but also possess a protective effect against thermally induced protein denaturation at physiological pH (pH: 6.2 to 6.5) (Ramalingam *et al*., 2010; Sangeetha *et al.,* 2011).

Correlations matrix

The correlations between antioxidants content (TP, TF, and CT) and antioxidant activities (DPPH, FRAP, H_2O_2 and BB) as well as anti-inflammatory activity of *O ficus-indica* L. seed extract were presented in Table 3. The correlations between the bioactive compounds and antioxidant activities reveal consistent positive associations. From Table 3, it can be seen the presence of a significant positive correlation ($p < 0.05$) between total polyphenols, total flavonoids, and condensed tannins with antioxidant activities measured by the DPPH, FRAP, H_2O_2, and BB as well as anti-inflammatory inhibition. These results suggest that these bioactive compounds may significantly contribute to the antioxidant activity of the hydro-soluble extract; these findings were in good agreement with a previous study by Fernández *et al*. (2010), who stated that the antioxidant activity of prickly pear grown in Spain was positively correlated with the phenolic compounds content of the extracts. Additionally**,** Rocha-Guzman *et al*. (2007) reported that the antiradical activity of acetone extract from seed and cactus pear fruit extract was highly correlated with the total polyphenols content. Ali *et al*. (2021) reported a high correlation between the extract of *Caragana brachyantha Rech.f*, of South Africa and bioactive compounds.

Table 3 demonstrates that TPC exhibits stronger correlations with the DPPH and PR tests, whereas Flavonoids display more moderate correlations. These differences may reflect the diverse antioxidant mechanisms present among the various bioactive compounds. Furthermore, the positive correlation between the bioactive compounds and the percentage inhibition of BSA reinforces the idea that

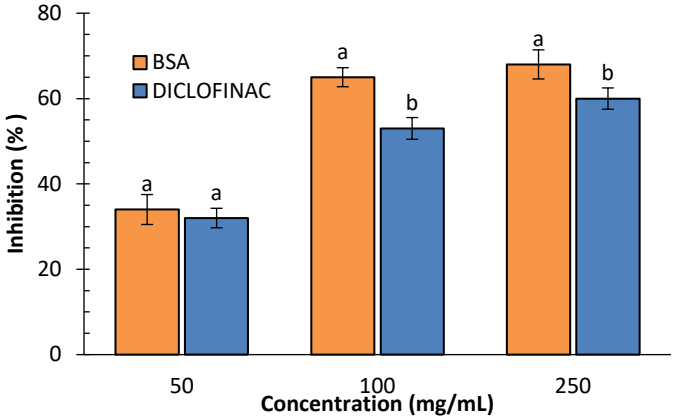

Figure 1. Percentage inhibition of heat-induced albumin denaturation by seed extract of *O ficus-indica* L. and diclofenac.

Table 3. Correlation matrix of bioactive compounds and both antioxidant and anti-inflammatory activities.

	Total polyphenols	Total flavonoids	Condensed tannins
DPPH radical scavenging activity	0.99	-0.89	-0.26
Ferric reducing antioxidant power	0.98	-0.71	0.04
Hydrogen peroxide scavenging activity	-0.70	0.98	0.82
β-carotene bleaching scavenging activity	-0.82	1.00	0.70
Anti-inflammatory activity	0.99	-0.99	0.99

these compounds may also be involved in modulating antioxidant activity against free radicals, thus contributing to the protection of proteins against oxidative damage.

CONCLUSION

This study underscores the significant antioxidant and anti-inflammatory properties of hydro-soluble *O ficus-indica* L. seed extract. Through various *in vitro* tests, the extract was found to be rich in total polyphenols, flavonoids, and condensed tannins, and demonstrated high antioxidant capacity across multiple assays. These findings suggest that the extract is a potent source of bioactive compounds with potential health benefits.

ACKNOWLEDGMENT

This work was funded by the Algerian Ministry of Higher Education and Scientific Research (PRFU project grant N° D00L01UN340120190001).

REFERENCES

Adesegun, S., Fajana, A., Orabueze, C.I. and Coker, H.A.B. (2007) Evaluation of antioxidant properties of *Phaulopsis fascisepala* C B Cl (Acanthaceae). *Evidence based Complementary and Alternative Medicine*, 6, 227-231.

Al Juhaimi, F., Kashif, G., Nurhan, U., Isam, A., Mohamed, A., El Fadil, E., Babikera, M.M.Ö. and Gbemisola, J.F. (2020) The effect of harvest times on bioactive properties and fatty acid compositions of prickly pear *(Opuntia ficus-barbarica A. Berger*) fruits. *Food Chemistry*, 303, 125-387.

Ali, L., Khan, S., Nazir, M., Raiz, N., Naz, S., Zengin, G., and Tareen, R. B. (2021). Chemical profiling, in vitro biological activities and Pearson correlation between phenolic contents and antioxidant activities of *Caragana brachyantha Rech.* f. *South African Journal of Botany*, 140, 189-193.

Ayoola, G. A., Adepoju, B. A. A., Obaweya, K., Ezennia, E. C. and Atangbayila, T. O. (2008) Phytochemical screening and antioxidant activities of some selected medicinal plants used in malaria therapy in Southwestern Nigeria. *Tropical Journal of Pharmaceutical Research*, 7, 1019-1024.

Azman, N.F.I.N., Azlan, A., Khoo, H.E. and Razman, M.R. (2019) Antioxidant properties of fresh and frozen peels of citrus species. *Current Research in Nutrition and Food Science Journal*, 7, 331-339.

Almeida, E. S., Carmona, P. O., Mendonça, S., Dias, A. C. B., Castellón, E. R., Cecilia, J. A., and Monteiro, S. (2024). The role of carotenes in preventing oxidation during palm oil processing: Adsorption studies. Industrial Crops and Products, 216, 118691

Barba, F.J., Putnik, P., Kovačević, D.B., Poojary, M.M., Roohinejad, S., Lorenzo, J.M. and Koubaa, M. (2017) Impact of conventional and non-conventional processing on prickly pear (*Opuntia spp*.) and their derived products: From preservation of beverages to valorization of by-products. *Innovative Food Science & Emerging Technologies*, 42, 58-69.

Blando, F., Albano, C., Jiménez-Martínez, C., & Cardador-Martínez, A. (2022). *Opuntia ficus-indica* [L.] Mill. and Other Species: Source of Bioactives and Their Molecular

Mechanisms of Action to Promote Human Health. In: Molecular Mechanisms of Functional Food Eds. Rocio Campos-Vega and B. Dave Oomah. John Wiley & Sons Ltd. https://www.wiley.com/en-us/Molecular+Mechanisms+of+Functional+Food-p-9781119804024

Brand-Williams, W., Cuvelier, M.E. and Berset, C.L.W.T. (1995) Use of a free radical method to evaluate antioxidant activity. *LWT-Food science and Technology*, 28, 25-30.

Cano, M.P., Gómez-Maqueo, A., García-Cayuela, T. and Welti-Chanes, J. (2017). Characterization of carotenoid profile of Spanish sanguinos and verdal prickly pear *(Opuntia ficus-indica, spp*.) tissues. *Food Chemistry*, 237, 612-622.

Cardador-Martínez, A., Jiménez-Martínez, C. and Sandoval, G. (2011) Revalorization of cactus pear (*Opuntia* spp.) wastes as a source of antioxidants. *Ciênciae Tecnologia de Alimentos*, 31, 782-788.

Chaalal, M., Louaileche, H., Touati, N., and Bey, M.B. (2013) Phytochemicals, in vitro antioxidant capacity and antiradical potential of whole and ground seeds of three prickly pear varieties: A comparative study. *Industrial Crops and Products*, 49, 386-391.

Chougui, N., Tamendjari, A., Hamidj, W., Hallal, S., Barras, A., Richard, T. and Larbat, R. (2013) Oil composition and characterization of phenolic compounds of *Opuntia ficus-indica* seeds. *Food chemistry*, 139, 796-803.

Dib, H. A. N. A. N. E., Beghdad, M. C., Belarbi, M. E. R. I. E. M., Seladji, M. and Ghalem, M. (2013). Antioxidant activity of phenolic compounds of the cladodes of *Opuntia ficus-indica mill.* from northwest Algeria. *International Journal of Medicine and Pharmaceutical Sciences*, 3(4), 147-158.

Fernández-López, J. A., Almela, L., Obón, J. M., and Castellar, R. (2010). Determination of antioxidant constituents in cactus pear fruits. *Plant Foods for Human Nutrition*, 65, 253-259.

García-Cayuela, T., Gómez-Maqueo, G., Guajardo-Flores, D., Welti-Chanes, J., and Pilar, C.M.P. (2019) Characterization and quantification of individual betalain and phenolic compounds in Mexican and Spanish prickly pear (*Opuntia ficus-indica L. mill*) tissues: A comparative study. *Journal of Food Composition and Analysis*, 76, 1-13.

Gómez-Maqueo, A., García-Cayuela, T., Jorge Welti-Chanes, J. and Canoa, M.P. (2019) Enhancement of anti-inflammatory and antioxidant activities of prickly pear fruits by high hydrostatic pressure: A chemical and microstructural approach. *Innovative Food Science and Emerging technologies*, *Elsevier*, 54, 132-142.

Han, X., Mi, Y., Ji, Y., Sun, M., Tang, H., Dong, F. and Guo, Z. (2024). A novel chitosan antioxidant bearing sulfhydryl group: Synthesis, characterization, and activity assessment. *International Journal of Biological Macromolecules*, 261, 129816.

Hamid, M. A., Yeap, C. H., Mustapha, W. A. W., Martony, O. and Fatmawati, F. (2024). Effects of different solvents on the antioxidant activity of several seaweed species from Semporna, Sabah, Malaysia. *Ilmu kelautan: Indonesian Journal of Marine Sciences*, 29(1).

Hihat, S.; Touati, N.; Sellal, A. and Madani, K. (2024) Response surface methodology: An optimal design for maximising the efficiency of microwave-assisted extraction of total phenolic compounds from *Coriandrum sativum* Leaves. *Processes*, 12, 1031. https://doi.org/10.3390/pr12051031

Jung-Woo, K., Jun-Kyu, S., Eun-Ji, K., Hyojeong, R., Hyoung, J.K. and Sun-Mee, L. (2016) *Opuntia ficus-indica* seed attenuates hepatic steatosis and promotes M2 macrophage polarization in high-fat diet–fed mice. *Nutritional research,* 36, 369-379.

Kandikattu, K., Kumar, P.B.R., Priya, V.R, Kumar, K.S. and Singh R.B.R. (2013) Evaluation of anti-inflammatory activity of *canthium parviflorum* by in vitro method. *Indian Journal of Research in Pharmacy and Biotechnology*, 1, 729-730.

Kartal, N., Sokmen, M., Tepe, B., Daferera, D., Polissiou, M. and Sokmen, A. (2007) Investigation of the antioxidant properties of *Ferula orientalis L*. using a suitable extraction procedure. *Food Chemistry*,100: 584-589.

Khatabi, O., Hanine, H., Elothmani, D. and Hasib, A. (2016) Extraction and determination of polyphenols and betalain pigments in the Moroccan Prickly pear fruits (*Opuntia ficus-indica*). *Arabian journal of chemistry,* 9, 278-281.

Kulisic, T., Radoni, A., Katalini, V. and Milos, M. (2004) Use of different methods for testing antioxidative activity of oregano essential oil. *Food Chemistry*, 85, 633-640.

Mena, P., Tassotti, M., Lucía, A.L., Nuncio-Jáuregui, N., Legua, P., Del Rioa, D. and Hernández, F. (2018) Phytochemical characterization of different prickly pear (*Opuntia ficus-indica L. Mill*.) cultivars and botanical parts: UHPLC-ESI-MSn metabolomics profiles and their chemometric analysis. *Food research international,* 108, 301-308.

Murad, S. A., Abd-Elshafy, D. N., Abou Baker, D. H., Bahgat, M. M., Ibrahim, E. A., Gaafar, A. A. and Salama, Z. (2023) Unveiling the anti-alzheimer, antioxidant, anti-Inflammatory, antiviral therapeutic functionality of polysaccharides extracted from *Opuntia Ficus*. *Egyptian Journal of Chemistry*, 66(5), 237-244.

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, 307-315.

Porter, L.J., Hrstic, L N. and Chan, B.G. (1986) The conversion of procyanidins and prodelphinidins to cyanidin and delphinidin. *Phytochemistry*, 25, 223-230.

Ramadan, M.F., Ayoub, T.E.M. and Rohn, S. (2021) *Opuntia spp*: hemistry, bioactivity and industrial applications. *Springer*, 3-11

Ramalingam, R., Madhavi, B.B., Nath, A.R., Duganath, N., Sri, E.U. and Banji, D. (2010) *In vitro* antidenaturation and antibacterial activities of *Zizyphus oenoplia*. *Der Pharmacia Lettre*, 2, 87-93.

Ramírez-Moreno, E., Cariño-Cortés, R., Cruz-Cansino, N.D.S., Delgado-Olivares, L., Ariza-Ortega, J.A., Montañez-Izquierdo, V.Y., Hernández-Herrero, M.M. and Filardo-Kerstupp, T. (2017) Antioxidant and antimicrobial properties of cactus pear (*Opuntia*) seed oils. *Journal of Food Quality*, ID 3075907, 1-8.

Ruch, R.J., Cheng, S.J. and Klaunig, J.E. (1989) Prevention of cytotoxicity and inhibition of intracellular communication by antioxidant catechins isolated from Chinese green tea. *Carcinogenesis*, 10, 1003–1008.

Rocha-Guzman, N.E., Herzog, A., Gonzalez-Laredo, R. F., Ibarra-Pérez, F. J., Zambrano-Galvan, G. and Galegos-Infante, J.A. (2007) Antioxidant and antimutagenic activity of phenolic compounds in three different colour groups on common bean cultivars (*Phaseolus vulgaris*). *Food Chemistry*, 103, 521-527.

Sakhraoui, A., Touati, N. and Hihat, S. (2023) Effect of time and temperature storage on the quality of unpasteurized prickly pear juice enriched with hydro-soluble *Opuntia ficus-indica* seeds extract. *Turkish Journal of Agriculture - Food Science and Technology*, 11, 1817-1824.

Salas, A. G., Bárcena-Gama, J. R., Ventura, J., Muñoz-García, C., Escobar-España, J. C., Crosby, M. M. and Hernandez, D. (2024) Bioaccessibility of condensed tannins and their effect on the physico-chemical characteristics of lamb meat. *Peer J*, 12, e17572.

Sakihama, Y., Cohen, M. F., Grace, S. C. and Yamasaki, H. (2002) Plant phenolic antioxidant and prooxidant activities: phenolics-induced oxidative damage mediated by metals in plants. *Toxicology*, 177, 67-80.

Sangeetha, M., Kousalya, M., Lavanya, R., Cherukuru, S., Chamundeeswari, D. and Maheswara, R. (2011) *In vitro* anti-inflammatory and anti-arthritic activity of leaves of *CleodendronInerme*. *Biological and Chemical Science*, 2, 822-827.

Sarikurkcu, C., Bektas, T.B, Daferera, D., Polissiou, M. and Harmandar, M. (2008) Studies on the antioxidant activity of the essential oil and methanol extract of *Marrubium globosum subsp*. *Globosum* (*lamiaceae*) by three different chemical assays. *Bioresource Technology*, 99, 4239-4246.

Tepe, B., Daferera, D., Sokmen, A., Sokmen, M. and Polissiou, M. (2005) Antimicrobial and antioxidant activities of essential oil and various extracts of *Salvia tomentosa Miller* (*Lamiaceae*). *Food Chemistry*, 90, 333-340.

Vermerris, W. and Nicholson, R. (2007) Phenolic compound biochemistry, Springer (Ed.), pp 24.

Yolmeh, M., Mohammad, B., Habibi, N. and Farhoosh, R. (2014) Optimization of ultrasound-assisted extraction of natural pigment from annatto seeds by response surface methodology (RSM), *Food Chemistry*, 155, 319-324.

Youssef, F.S. (2021) Genus *Opuntia*: A golden source of compounds with anti-inflammatory potential. *Opuntia spp*.: *chemistry, Bioactivity, and Industrial Applications*, 411-422.

Zeghad, N., Ejaz, A., Belkhiri, A., Van Hoed, V.Y. and Demyeer, K. (2019) Antioxidant activity of *Vitis vinifera*, *Punica granatum*. *Citrus aurantium* and *Opuntia ficus-indica* fruits cultivated in Algeria. *Heliyon*, 5, e01575.