Phytochemical screening and Antioxidant activity of *Opuntia ficus-indica* seeds from Algeria

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Abstract: *Opuntia ficus-indica* (L.) Mill. is the Cactaceae plant with the greatest economic relevance in the world. The biologically active compounds isolated from plants are known to be efficient as antibacterial and antioxidants. The aim of this study was to determine the polyphenolic, profile and characterize the antioxidant capacity of seeds of Algerian prickly pear cultivar. The determination of total phenolic, flavonoid and tannin contents of *Opuntia ficus-indica* seeds were carried out by the Folin-Ciocalteu, the aluminum trichloride and the polyvinyl polypyrrolidine spectrometric methods. Antioxidant activities of seeds extract were assessed using free radical scavenging assay DPPH, Ferric Reducing Antioxidant Potential FRAP and *β*-Carotene bleaching assay. The present study shows that seeds are rich in total phenolic and flavonoids contents 38.99±0.10 mg EAG/g DW and 31.58±2.00 mg CE/g DW, respectively. The content of total tannins was estimated at 2.8±0.01 mg CE/g DW. The tannins extract showed the most important inhibition of DPPH radical activity (IC_{50}= 10±0.008 µg/mL) higher even compared with standard antioxidants ascorbic acid and Trolox. Also a stronger *β*-Carotene bleaching inhibition capacity (IC_{50}= 0.46±0.04 mg/mL) is observed. In this context, the results presented deduce that these seeds are a promising source of phenolic compounds. Biological studies are needed to elucidate and conclude the effectiveness of these plants, which can be a challenge for new food and cosmetic products.

Keywords: *Opuntia ficus-indica*, seeds, phenolic compounds, antioxidant activity.

Introduction

There has been a recent trend in consumer demand for foods with higher nutritional value, as well as with health benefits, which has spawned a new category of ‘functional foods’. The health benefits include disease prevention (Ghazi et al. 2015).

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Cactus plant (*Opuntia ficus-indica*) of the Cactaceae family, grows native in Mexico and has been used since many of years as source of food. Genus Opuntia contains about 1500 species of cactus which are widespread in Africa, Mediterranean countries, Northern Mexico, Southwestern United States, and other areas (Salama et al. 2020). This plant is mainly known for its edible, nutritionally rich sweet fruit, but cladodes are also consumed, mainly in Mexico, which is the country with the largest area under cultivation and also the largest producer (FAO 2018). They grow also throughout Algeria where they are used mainly for human food, exclusively as fresh fruit, but also for livestock forage and planted as ornamentals or for fencing.

Opuntia species are used to treat chronic diseases such as, diabetes, obesity, cardiovascular diseases, and cancer (Santos-Díaz et al. 2017). Pharmacological studies on cactus fruit revealed a wide range of biological activities, including antioxidant, anticancer, anti-inflammatory and anti-allergic activities (Benayad et al. 2014; Ammar et al. 2015; Barba et al. 2017; Fiad et al. 2020).

The fruit of *Opuntia ficus-indica* provide interesting sources of functional compounds, including total phenols, flavonoids, carotenoids, dietary fibers, betalains, taurine and linoleic acid that exhibit several biological activities (Khatabi et al. 2011). These compounds are valued for their contribution to a healthy diet and also as ingredients for designing new foods. Being high in nutritional and bioactive phytochemicals, cactus pear fruit can be used both as a potential source of natural antioxidants and as a direct functional food (Kolniak-Ostek et al. 2020).

Many reports have expounded a strong link between the phenol content and the antimicrobial, antioxidant activities of polyphenol extracted from *Opuntia* spp. (Kuti 2004; Castellanos-Santiago and Yahia 2008; Anwar and Sallam 2016).

The efficacy of the plants in the treatment of different human diseases has been attributed to the presence of different active compounds, principally phenolic derivatives. These compounds have been well-reported to exert antioxidant properties. They may prevent the damage caused by reactive oxygen species (Algieri et al. 2016).

The edible part of the fruit contains a relatively large number of seeds, which were effectively demonstrated to be rich in the health-promoting polyunsaturated fatty acids and vitamins and may potentially be included in animal and human diets (Ramadan & Mörsel 2003; Loizzo et al. 2020). Hence, the *Opuntia ficus-indica* plant parts and by-products had recently been

attracting a lot of research interest and perhaps integral to the detection of novel and natural bioactive compounds.

The literature contains much information concerning the cactus pear, describing the chemical composition of the pulp, peel and seeds (El Kossori et al. 1998; Felker, Soulier, Leguizamon, & Ochoa 2002; Matsuhiro et al. 2006; Kolniak-Ostek et al. 2020), the composition of oil (Chougui et al. 2013; Salama et al. 2020), the antioxidant, anti-inflammatory and anti-ulcerogenic properties of the pulp (Ahmed et al. 2005;Galati et al. 2001; Livrea & Tesoriere 2006) and, more generally, the nutritional significance of Opuntia sp. (Stintzing, Schieber, & Carle, 1999). However, to our knowledge among all this literature, seed has been the less studied part.

This work based its interest on Algerian prickly pear cultivar seeds and aimed to quantify the phenolic compounds contained in the hydromethanolic extracts of *Opuntia ficus-indica* seeds (OFIS), and to evaluate their antioxidant capacities by DPPH° assay, iron reduction and β-Carotene bleaching inhibition capacity, in order to show possible differences in antioxidant content and improve the knowledge of bioactive compounds, which may be helpful for therapeutic and pharmaceutical applications.

Material and methods

Plant material

Opuntia ficus-indica fruit were collected from Aouchba (North-West of Tlemcen city, Algeria) and identified by Pr. Benabadji.N, Tlemcen University (Algeria). Seeds were isolated from the fruit, dried and ground as described by Nassar (2008).

Extraction of chemical compounds from OFIS

Total phenolic compounds

The OFIS phenolic extract was obtained according to the method of Jimoh et al. (2010) with slight modifications. Briefly, 10 g of the ground OFIS were extracted with methanol:water, 80:20 (v/v) by maceration at room temperature for 24 hours. Then the extracts were filtered through filter paper under vacuum. The filtrate was concentrated to dryness under reduced pressure at 45 °C, and then dissolved in 10 mL of methanol. The methanolic extract was stored at 4 °C, for further investigations.

Total flavonoid compounds

10 g of dried material were extracted with 100 mL of methanol (MeOH) and 5 g of carbonate of Calcium by boiling for 1 h (Dauguet & Foucher 1982). After filtration, the MeOH was evaporated under reduced pressure. Subsequently, recover the dry extract with 50 mL of boiling water. The aqueous extract was filtered and then fractionated by liquid – liquid extraction, first with Diethyl Ether, Ethyl acetate and then with n-butanol, using a separating funnel. All the fractions were concentrated and kept at 4 °C.

Total Tannin compounds

Tannins extraction from OFIS (5 g) was extracted at 4 °C using 200 mL of a mixture of acetone – water (25/45, v/v) for 4 days (Bruneton 1999). The extracts were filtered under vacuum through filter paper, and acetone was evaporated under reduced pressure. Subsequently, the dichloromethane (2 × 25 mL) was used for the extraction of lipids and pigments from the aqueous extracts using a separating funnel. Afterward, the aqueous phase was extracted with 25 mL of ethyl acetate. This process was repeated 4 times. After filtration, the organic phases (ethyl acetate) containing tannins were recovered and concentrated to dryness under vacuum using a rotary evaporator. The residues obtained were weighed and preserved until uses.

Determination of total phenolic and tannin contents

Total phenolic contents in the extract were determined using the Folin–Ciocalteu reagent according to the modified method of Singleton and Rossi (1965).The content of phenolic compounds was determined with reference to standard curve determined with gallic acid, and expressed as mg gallic acid equivalents /g dry matter (mg GAE/g DM).

Using the same extract, the tannin contents were estimated after treatment with polyvinyl polypyrrolidine (PVPP) that served as precipitate agent (Siddhuraju and Manian2007). 1 mL of tannin-containing phenolic extract was mixed with 1 mL of distilled water and 100 mg of PVPP. The mixture was vortexed and kept at 4 °C for 15 min. Then the sample was centrifuged at 1700 g for 10 min at room temperature and the supernatant was collected. The phenolic content of the supernatant was measured as mentioned above and expressed as the content of non-tannin phenolics in dry matter basis. The tannin content of the sample was calculated as follows:

(Tannins (%) = Total phenolics (%) − Non-tannin phenolics (%)).

Determination of total flavonoid contents

Total flavonoid contents were determined according to Djeridane et al. (2007). In brief, 1 mL of extract was mixed with 1 mL of methanolic $AlCl_3$ (2%). After incubation at room temperature for 15 min, the absorbance was measured at 430 nm. The content of flavonoids was determined with reference to standard curve determined with catechin, and expressed as mg catechin equivalents /g dry matter (mg CE/g DM).

***In vitro* evaluation of antioxidant activity**

DPPH Free-Radical-Scavenging Assay

The hydrogen atom or electron donation abilities of some pure compounds were measured by the bleaching of a purple colored methanol solution of the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical (Sanchez-Moreno et al. 1998). 50 µL of various concentrations of the extracts in methanol was added to 1950 µL of a methanolic solution of DPPH at 6.34 10^{-5} M. After a 30 min incubation period at room temperature, the absorbance was read against a blank at 515 nm. DPPH free radical scavenging activity in percentage (%) was calculated using the following formula:

$$\text{DPPH scavenging activity (\%)} = (A_{blank} - A_{sample} / A_{blank}) \times 100$$

Where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound), a sample is the absorbance of the test compound.

Extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotted of inhibition percentage against extract concentrations. The ascorbic acid (AA) and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were used as positive control.

Ferric Reducing Antioxidant Potential (FRAP)

After adding 1 mL of each extract at different concentrations with 2.5 mL of 0.2 M phosphate buffer at pH= 6.6 and 2.5 mL of a 1% potassium ferricyanide solution, the obtained mixture was incubated for 20 min at 50 °C, and then 2.5 mL of 10% trichloroacetic acid was added to stop the reaction. The mixture was centrifuged at 650 g for ten minutes at room temperature and 2.5 mL of the supernatant were added to 2.5 mL of distilled water and 0.5 mL of 0.1% iron chloride. The absorbance was read at 700 nm against a blank. The results make it possible to calculate the effective concentration (EC_{50}), which is the extract concentration

corresponding to an absorbance equal to 0.5, the linear regression curve (optics density as a function of the different concentrations). The extract activity was finally compared with that of the positive control ascorbic acid (AA) and Trolox (Oyaizu 1986).

β-Carotene bleaching inhibition capacity

The capacity of extracts to inhibit the β-carotene bleaching was determined according to Koleva et al. (2002) with minor modifications. Two mg of β–carotene were dissolved in 20 mL chloroform, and 4 mL of this solution were added to linoleic acid (40 mg) and Tween 40 (400 mg). Chloroform was evaporated under vacuum at 40 °C and 100 mL of oxygenated water were added. An emulsion was obtained by vigorously shaken, an aliquot (150 µL) of which was distributed in 96 well microtitre plate and methanolic solutions of the test samples (10 µL) were added. Three replicates were prepared for each extract concentration. The microplate was incubated at 50 °C for 120 min, and the absorbance was measured at 470 nm using a EAR 400 microtitre reader (Multiskan MS, Labsystems). Readings were performed both immediately (t 0 min) and after 120 min of incubation. The antioxidant activity of the extracts was evaluated in terms of bleaching inhibition of the β carotene using the following formula:

$$\beta\text{-carotene bleaching inhibition (\%)} = [(S - A_{120})/(A_0 - A_{120}) \times 100]$$

Where A_0 and A_{120} are the absorbance of the control at 0 and 120 min, respectively, and S the sample absorbance at 120 min. The results were expressed as IC_{50}value (mg/mL).

Statistical analysis

All evaluations were performed at least in triplicate. Data were expressed as means ± standard deviation (SD).

Results and discussion

Determination of total phenolic, flavonoid and tannin contents

Recently, the research has been directed towards natural antioxidants in the seeds. Indeed, seeds from Opuntia sp. were shown to be rich in polyphenols, flavonoids and tannins, the concentrations of those molecules being always higher than in the fruit pulp (Cardador-Martínez et al. 2011; Morales et al. 2012).

Preliminary phytochemical screening of OFIS extracts revealed the presence of various bioactive components like tannins, phenols, flavonoids and Alkaloids. Evidences have shown that phenolics constitute the main powerful antioxidant compounds (Franco et al. 2017). For that, total phenolic, flavonoids and tannins contents of methanolic extract from OFIS were assessed by the spectrophotometric methods. The results obtained are expressed in milligrams of gallic acid equivalent per gram of dry matter (mg GAE/g DW) (Table 1).

Table 1. Total phenolics, flavonoids and tannins content of *Opuntia ficus-indica* seeds.

Chemical constituent	Phenolic (mg GAE/g DM)	Flavonoids (mg CE/g DM)	Tanins (mg GAE/g DM)
Opuntiaficus-indica Seeds	38.99±0.10	31.58±2.00	2.8±0.01

mg GAE/mg DW: mg of gallig acid equivalents per g of dry Matter. mg CE/mg DW: mg of catechine equivalents per g of dry Matter. The data are displayed with mean ± standard deviation of triplicate.

Results of total phenolic compounds revealed that OFIS methanolic extract contained an important amount of polyphenols (38.99±0.10mg GAE/g DW), where flavonoids constitute the major class (82% of total phenolic contents). Tannins were found to be minor as compared to flavonoids (7.17% of total phenolic contents). Results of phenolics content are higher than those obtained by Tlili et al. (2011) for the Algerian Opuntia seeds who that shown values of phenolics content 268.4 mg GAE /100g. In other hand, Chougui et al. (2013) have obtained 48–94 mg GAE/100g for total phenolics as well as flavonoids (1.5–2.8 mg QE/ 100 g) and tannins amounted 4.1–6.7 mg CE/100 g. These differences may be due to cultivar and genetic factors, growth conditions, as well as harvesting time, degree of ripeness or fruit processing, and above all, the determination methods (De Wit et al. 2018).

On the other side, the total phenolic contents calculated in mg EGA/kg DW extracted seeds from 8 different cultivars of prickly pear studied by De Wit et al. (2018) were ranging from 74.86 mg/kg to 291.46 mg/kg. Their results were slightly higher than those found by Kolniak-Ostek et al. (2020) in extracts seeds from Spanish cultivars.

The results obtained show that the composition of prickly pear seeds can vary among cultivars, varieties and crop environmental factors, among others (Ramírez-Moreno et al. 2017). In addition, most studies have shown that extraction solvents and processing methods used could affect biological activity, yield and phenolic compound profile of tested extracts (Torres et al. 2010; Abou-Elella and Ali 2014).

Assessment of antioxidant activity

Antioxidant activity is one of the major mechanisms by which fruit and vegetables provide health benefits. The high amounts of polyphenols, which show strong antioxidative properties attributed to their ability to scavenge free radicals and to chelate metal ions involved in their production, contribute to the strong antioxidant activity of prickly pear seeds (El-Hawary et al. 2018). The evaluation of the antioxidant power of the extracts of OFIS was determined by three in vitro methods, the trapping of the free radical DPPH°, the iron reduction and the β-Carotene bleaching inhibition capacity.

DPPH Free-Radical-Scavenging Assay

The effect of antioxidant on DPPH radical scavenging was thought to be due to their hydrogen donating ability or radical scavenging activity. When a solution of DPPH is mixed with a substance that can donate a hydrogen atom, it then leads to a loss of this violet color (Haddouchi et al. 2018). The results obtained for the positive controls, ascorbic acid, Trolox and for the extracts were expressed as an IC_{50} (mg/mL) values of the different extracts of OFIS (Table 2).

Table 2. IC_{50} (mg/ml) values of DPPH Free-Radical-Scavenging assay of *Opuntia ficus-indica* seeds extracts

Extracts	DPPH IC_{50}(mg/ml)
PT	0.04 ±0.007
Flv ED	0.18±0.01
Flv EA	0.09±0.008
Flv NB	0.06±0.004
Tanins	0.01±0.008
AA	0.18±0.08
Trolox	0.159±0.04

PT: total phenolic, Flv ED: diethyl ether, Flv EA: ethyl acetat, Flv NB: n-butanolic, AA: ascorbic acid. The data are displayed with mean ± standard deviation of triplicate. Mean values followed by different superscript in a column are significantly different ($p<0.05$).

As shown in table 2, the tannins extract was the most potent of all studied extracts (IC_{50}= 0.01±0.008 mg/mL). This value was higher than that of the standard antioxidants, namely ascorbic acid and Trolox whose IC_{50} values are of the order of 0.18±0.08 and 0.159±0.04 mg/mL respectively. The total phenolic, the n-butanol and ethyl acetate flavonoids extracts of OFIS had closer antioxidant activities with IC_{50} about 0.04 ± 0.007, 0.06±0.004 and 0.09±0.008 mg/ mL respectively. However, a similarity was observed for diethyl ether

flavonoids extract and ascorbic acid having respective IC_{50} of 0.18 ±0.01 and 0.18±0.08 mg/mL. Tannins extract activity of seeds surpassed that of the flowers and the cladodes of *Opuntia ficus-indica* (Dib et al. 2013; 2014). Tannins are known to inhibit lipid peroxidation in vitro and have the ability to scavenge free radicals important in cellular pro-oxidant states. Most of the activities of tannins, including their free radical-scavenging capacity, largely depend on their structure and degree of polymerization (Okuda 2005; Jerez 2007).

Ferric Reducing Antioxidant Potential (FRAP)

FRAP assay was used by several authors for the assessment of the antioxidant activity of various food product samples (Halvorsen et al. 2006; Pellegrini et al. 2003).

The Iron reduction is a method based on the reduction capacity of Fe3+ present in the $K3Fe(CN)_6$ complex into Fe^{2+}. Figure 1 represents the reducing capacity of ascorbic acid, Trolox and different extracts of OFIS. This capacity increases as a function of the concentration of the extracts. At a concentration of 5 mg/ml, it is observed that the extract from tannins has the highest absorbance (1).

The efficiency of iron reduction is inversely proportional to the EC50 value, it is of the increasing order according to the following classification: Ascorbic acid > Trolox > tannins > n-butanol flavonoids > ethylacetat flavonoids > total phenolic > diethylether flavonoids.

β-Carotene bleaching inhibition capacity

In the β-Carotene model system, its undergoes rapid discoloration in the absence of an antioxidant, which results in a reduction in absorbance of the test solution with reaction time. The presence of antioxidant avoids the destruction of the β -carotene conjugate system and the orange color is maintained (Seladji et al. 2014). The obtained results are summarized in table 3.

The antioxidant activity of tannins extract was very highly compared with the different extracts of OFIS with IC_{50} 0.46±0.04 mg/mL. This IC_{50} is closer than that IC_{50} of positive control gallic acid 0.43±0.008 mg/ mL but lower than that Trolox which IC_{50} 0.24±0.003 mg/mL. The β-carotene bleaching test confirms the powerful and very promising potent activity of tannins extract of OFIS as a source of natural antioxidants.

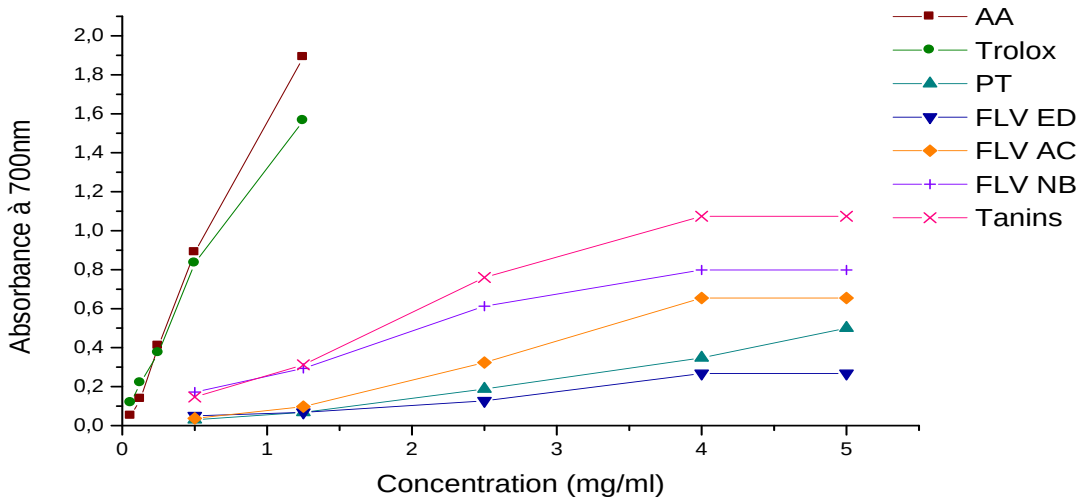
Furthermore, significant differences in antioxidant activity have also been recorded for ground seeds compared to whole prickly pear seeds which were attributed to their high total phenol composition (Morales *et a*l. 2014). These activities depended mainly on the type of

solvent used for extraction (Nguyen et al. 2017), the extraction method as well as the geographical location of the plant and the harvest season (Chaouche et al. 2020).

Table 3. IC_{50} (mg/ml) values of β carotene-linoleic acid assay of *Opuntia ficus-indica* seeds extracts

Extracts	β-caroteneIC$_{50}$(mg/ml)
PT	0.81 ±0.07
FlvED	2.85±0.07
Flv EA	0.82±0.07
Flv NB	2.58±0.08
Tanins	0.46±0.04
GA	0.43±0.008
Trolox	0.24±0.003

PT: total phenolic, Flv ED: diethyl ether, Flv EA: ethyl acetat, Flv NB: n-butanolic, GA: gallic acid. The data are displayed with mean ± standard deviation of triplicate. Mean values followed by different superscript in a column are significantly different ($p<0.05$).



AA : acide ascorbique ;Trolox: 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, PT : total phenolic ; EDFLV: diéthyletherflavonoïds, EAFLV: ethylacetatflavonoïds, FLVNB: n-butanolflavonoïds.

Figure 1. Total reducing power of *Opuntia ficus-indica* seeds extracts

Conclusion

This research intends to value prickly pears of local origin; the results will provide specific information about the quantification of phenolic compounds and the antioxidant power of Algerian *Opuntia ficus-indica* seeds. From the results obtained, we noticed that the seeds of *Opuntia ficus-indica* are an excellent source of nutrients and health-promoting substances due

to the high content of phenolic compounds and a significant antioxidant power of tannins compared to other extracts and better than the positive controls. The study of the composition of this seeds constitutes an advance in the knowledge of their properties and in the elaboration of derived products. Therefore, seeds from prickly pear could be regarded as a potential health-promoting functional ingredient and used as a food supplement and possibly use them in the cosmetic and pharmaceutical industries.

Conflicts of Interest

No potential conflict of interest was reported by the authors.

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