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Research Article

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Evaluation of the nutritional value and antioxidant activity of *Opuntia ficus indica* seeds in the western region of Algeria

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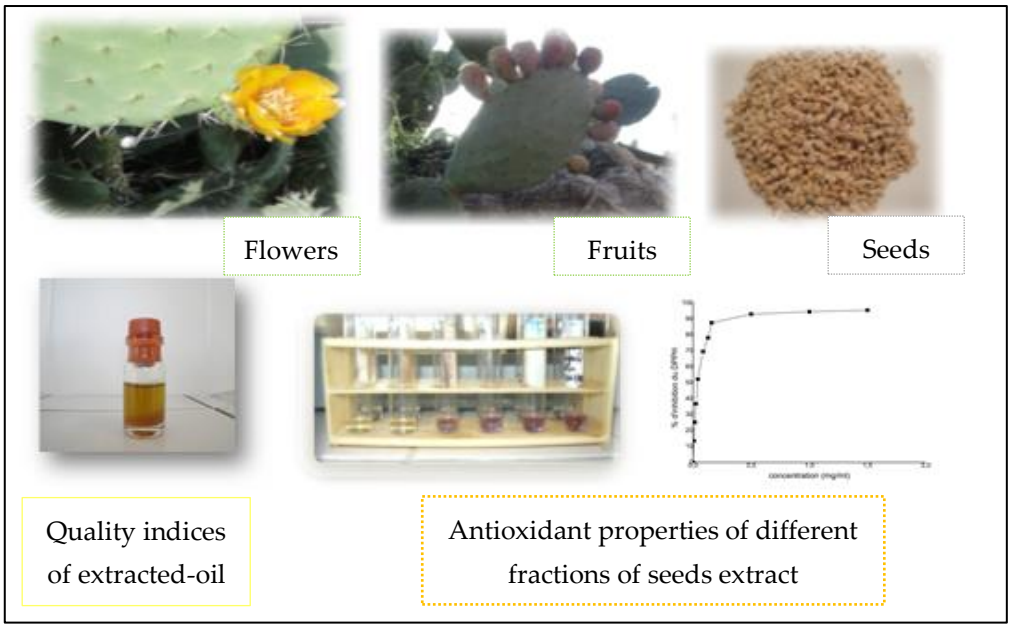
Highlights

- *Opuntia ficus indica* (OFI) seeds have an intriguing nutritional profile.
- Antioxidant activity of OFI seeds were examined.
- Quality indices of OFI seeds oil were determined.
- OFI seeds can be used in nutrition.

Graphical Abstract



Abstract
The *Opuntia ficus indica* (OFI), commonly known as "prickly pear" is a Cactaceae native to Central America that is well suited to arid and semi-arid areas such as Algeria. In recent decades, interest in this long-ignored cactus has increased. Different parts including (cladodes, fruits, flowers) of this plant have been studied, but little work has been devoted to the seeds, which are considered as by-products. It is in this context our research was conducted, it aimed to establish the nutritive value and antioxidant power of these seeds. The study of seed composition showed high crude fiber content estimated at 71.91% and significant levels of fat and protein 10.19% and 9.19%, respectively. The best oil yield was obtained after 8-hour of oil extraction. Given that its density and refractive index were calculated to be 0.916 and 1.470, respectively, this oil is of higher grade. The saponification and acidity were in the range of 161.31 and 1.74, respectively. The total polyphenols were about 1.110 mg EQG / g of DW. The antioxidant activity was revealed by the DPPH free radicals scavenging method. The obtained results were assigned the highest antioxidant activity with flavonoids over 90% at a concentration of 0.5 mg / mL for both fractions (acetate and n -butanol), their EC 50 values are about of 0.089 mg / mL and 0.063 mg / mL, respectively, the latters are close to the EC 50 of ascorbic acid. Thus, OFI seeds may be of interest to the cosmetics industry for its beneficial properties.

Keywords: *Opuntia ficus indica*, seeds, oil, nutritive value, antioxidant activity.

1. Introduction

The prickly pear, belonging to the family cactaceae, scientifically named as *Opuntia ficus indica* L., is native to Mexico (Mulas and Mulas, 2004). This plant with high economic importance is still very little exploited. The plant can be used in agri-food, pharmaceutical and cosmetics (Arba, 2009). In the last decades, the research had been focused on natural antioxidants in the seeds. It has been proven that seeds from *Opuntia ficus indica* are rich in polyphenols, flavonoids and tannins, which means it contains more bioactive molecules than the fruit pulp (Cardador-Martínez et al., 2011; Morales et al., 2012).

Prickly pear seed oil is a natural reservoir of bioactive molecules which make the oil a nutritionally interesting product. This oil contains a very high content of linoleic acid, ranging from 49.7% to 62.1% (Chougui et al., 2013; Ennouri et al., 2005; Ennouri et al., 2006a; Labuschagne and Hugo, 2010; Matthäus and Özcan, 2011; Ramadan and Moersel, 2006; Yeddes et al., 2012; Ortega-Ortega et al., 2017). This composition of oil shows several interesting properties (Ennouri et al. (a), 2006), it improves or lower cholesterol levels and possess hypoglycemic effects (Ennouri et al., 2006 (a); Ennouri et al., 2006 (b); Chougui et al., 2013). Therefore, the present work was aimed to determine the antioxidant activity and quality of polyphenols and oil extracted from *Opuntia ficus indica* seeds.

2. Materials and Methods

2.1. Plant material

Prickly pear fruits were picked in December 2010 from Beni-Saf (more than 45 km from Tlemcen in the Northeast direction) between Rachgoun and Hdahda (near Oulhaca). The fruits were at the end of maturity and in sufficient quantity for all the experiments. Fruits were

nuanced colors from yellow to pink or red. The seeds were well washed, dried and then stored in a jar away from light and humidity

2.2. Determination of primary metabolites

Procedures described by AOAC (1990) were used to determine moisture, crude fiber, ash and protein Kjeldahl nitrogen. Fat and carbohydrates were determined following ISO 659 and Dubois et al, (1956), respectively. All experiments were performed in triplicate to ensure the reproducibility of the results.

2.3. Extracted oil quality indices

The oil was extracted with n-hexane in a Soxhlet apparatus. The extract was evaporated in vacuum. The oil was collected in a flask and stored for further analysis. Relative density, refractive index, acidity and saponification number of oil were analyzed according to the method described by AOAC (1990).

2.4. Phytochemical screening

Phytochemical tests for the presence of alkaloids, flavonoids, tannins, saponins, starch, sterols and triterpenes, reducing compounds were carried out according to Harborne (1973), Sofowara (1993), Trease and Evans (1987) and Bruneton (1999), respectively in aqueous, ethanolic and etheric extracts.

2.5. Determination of secondary metabolites

Polyphenols were extracted by two independent extractions with methanol and acetone as solvents with (80/20) and (70/30), respectively. The content was determined according to (Brune et al. 1991) and (Blahova et al., 2004). Flavonoid-content was evaluated by spectrophotometer according to a method based on the formation of a complex flavonoid aluminium (Djeridane et al., 2006). The ethyl acetate and butanol fractions containing flavonoids were prepared according to Bekkara et al.(1998). Condensed tannins content was estimated using vanillin-HCl method as reported by Swain and Hillis (1959) and non-condensed tannins content were estimated using the complex ($FeCl_3$/HCl) following (Mole et Waterman, 1987). The results of Polyphenols and flavonoid content were expressed in mg gallic acid and catechin equivalent per 100 g dried weight (mg CE/ 100 g DW), respectively.

2.6. DPPH radical-scavenging activity

Evaluation of the scavenging activity against the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) was measured according to the method described in Sanchez-Moreno et al. (1998). A volume of 50 µL of various concentrations of each extract was mixed with 1950 µL of DPPH methanol solution (0.025% w/v). The mixture was shaken vigorously and incubated at room temperature. The absorbance of the samples was measured by spectrophotometer at 515 nm. Ascorbic acid as DPPH scavenging compound was used as positive control. DPPH-free radical scavenging activity was expressed as a percentage (%) according to the following equation:

%DPPH scavenging activity = [(A blank – A sample)/ A blank] x 100

Where A blank is the absorbance of the control, A sample is the absorbance of the compound tested.

3. Results and discussion

3.1. Primary metabolites

Based on the recorded data (Table 1), it was noted that the amount of moisture in the seeds does not exceed 8%. Moisture content is an important factor influencing the quality of oil

extracted. Reduced water content may be a factor in oils' decreased rancidity and oxidation. (De Wit et al., 2017). The protein content of seeds is about 9.19 g/100 g DW these proteins could be used as healthy ingredients in nutraceutical foods. The moisture and protein content is in line with Nounah et al. (2020) who found 8.1% and 7 g/100 g DM, respectively. The oil rate has been estimated to be 10.19% which is close to other findings (Ramadan and Mörsel, 2003; Ennouri et al., 2005). The variety, the color of the fruit and the region of cultivar can explain this difference (Chougui et al., 2013).

Loizzo et al., (2019), supported conventional seeds oil extraction. They showed that ultrasound-assisted extraction offers lower performance (amount of bioactive compounds from the oil) than extraction using a Soxhlet apparatus. Nevertheless, all these findings are lower than crude oil yields found in fruits of Turkish origin with contents amounting up to 14.4% of DM (Coskuner and Tekin, 2003; Matthäus and Özcan, 2011). In terms of yield, OFI seeds are considered low compared to other oil seed crops. Indeed, high amounts were recorded in cotton seeds, soybean and olive ranging from 15 to 25% (Matthäus and Özcan, 2006).

The ash percentage is in agreement with Boukeloua et al., (2012) estimated at 1.74% and 1.46%, respectively.

Few works have been devoted to the fiber and sugar content of OFI seeds. However, considerable fiber content (71.91%) was noted, which is higher than other results (Özcan and Juhaimi, 2011; El Kossori et al., 1998). Therefore, these OFI seeds are a good source of dietary fiber.

Table 1. Chemical Composition of *Opuntia ficus indica* Seeds

Component	Content (mg/100 g dry weight)
Moisture content	7.97%
Oil	10.19%
Ash	1.74%
Fibers	71.91%
Protein	9.19%
Carbohydrates	Not identified

3.2. Oil quality indices

In order to identify the quality of OFI seeds oil, physico-chemical indices were determined and the results are shown in Table 2.

Table 2. Physico-chemical characteristics of OFI seeds

Properties	Values
Acid value (g of I_2/100 g of oil)	1.74
Saponification index (mg of KOH/g of oil)	161.31
Density	0,916
Refractive index	1.470
Oil Color	Greenish yellow
Odor	Slightly fruity

3.2.1. Density index

The density index is a physical criterion that allows oil control extracted. The obtained results were 0.916. This value is close to that found by El Manoubi et al., (2009) and Ennouri et al., (2005) who found respectively 0.904 and 0.903 ± 0.002. Subsequently, this OFI seeds oil is pure.

3.2.2. Refractive index

The refractive index, which is also an important criterion of oil purity, is proportional to the molecular weight of the fatty acids. It varies interestingly depending on the degree of lipid unsaturation and can give us an idea of the predominance of one or more such unsaturated fatty acids in oil (Ollë, 2002). The refractive index was estimated at 1.470, which is similar to the results found El Manoubi et al., (2009) and Ennouri et al., (2005). Likewise, these results confirm that the extracted oil is pure.

3.2.3. Saponification index

The saponification index is about 161.31. This value is lower than that found by Boukeloua et al., (2012) estimated at 177.30. This difference in results is explained by a slight degradation of lipids, this oil is therefore rapidly degradable.

3.2.4. Acid index

The acid index expresses the degree of purity and freshness of the oil, but also on its degree of alteration (Ollë, 2002). The acid value was 1.74, which is close to that of found by El Manoubi et al. (2009) in the order of 1.27±0.005; this slight difference can be explained by the fact that the fatty acids present in the oil are alterable compounds. Kolniak-Ostek et al. (2020) identified 13 different fatty acids in prickly pear seeds from seven Spanish prickly pear cultivars. Linoleic acid was the predominant fatty acid found in the two varieties “Nopal ovalado” and “Nopal espinoso”. Indeed, Yeddes et al. (2012) found that linoleic acid was the major compound followed by oleic acid and palmitic acid for two extraction processes soxhlet and supercritical carbon dioxide (SC-CO_2). However, the latter claimed that the cultivated type could extract unsaturated fatty acids more effectively. Filip et al., (2011) reported that the linoleic acid level in OFI seed oil is ranging from 53.5% to 70.29%, which is higher than in sunflower oil. As a precursor of arachidonic acid, linoleic acid has long been acknowledged for its hypocholesterolemic effect and inhibitory properties toward colon cancer metastatic cells (Soel et al., 2007). Linolenic acid is known to be beneficial for variety of health disorders including cardiovascular diseases, inflammatory conditions, autoimmune disorders and diabetes (El-Mostafa et al., 2014).

3.3. Secondary metabolites

Phenolic compounds are a family of organic molecules commonly present in the plant kingdom (Cartea et al., 2011). Phenolic compounds have antioxidant properties due to their ability to

trap free radicals and reactive oxygen species which can initiate the radical process (Sökmen et al., 2012).

The alkaloids, flavonoids and tannins were positively detected with phytochemical tests as mentioned in Table 3. The sterols and triterpenes were weakly detected.

Table 3. Phytochemical screening of OFI seeds in different fractions.

Family of compound	**Aqueous extract**	**Ethanol extract**	**Ether extract**
Alkaloids		++	-
Flavonoids	++		
Tannins		++	-
Saponosides	-		
Starch	+		
Sterols et triterpenes	+		
Reducing compounds	+		

The queous acetone extraction expressed a better yield than the aqueous methanol with 1.110 mg EQG/g and 0.960 EQG/g of DW, respectively (Table 4). Our results confirm those of Yu and Dahlgren (2000), who points out that the solvent aqueous acetone, is more effective than aqueous methanol. Indeed, the efficiency of the extraction is related to the particle size, the solvent used but also the type of plant tissue or the studied part of the plant (Hayouni et al., 2007). In addition, the concentration of polyphenols decreases with the stage of maturity (Kennedy et al., 2000). However, our OFI seeds come from ripe fruits.

Table 4. Phenolic compounds of *Opuntia ficus indica* seeds.

Phenolic compounds	**Content (DW)**
Total phenolics extracted with aqueous methanol	0.960 mg EQG/g
Total phenolics extracted with aqueous acetone	1.110 mg EQG/g
Flavonoids	0.658 mg EQC/g
Condensed Tannins	0.07 mg EQG/g
hydrolysables Tannins	0.106 mg EQG/g
Alkaloids	0.12 mg EQG/g

In the other hand, Nounah et al. (2020) showed that the total content of phenolic compounds changed with the roasting time. The total phenolic compounds concentration decreased from 225.9 mg GAE/100 g to 188 mg GAE/100 g of DW during 20 min of roasting. This decline might be brought about by the heat treatment's destruction of phenolic chemicals found in nature.

Moreover, Chbani et al. (2020) deduced that mild roasting of the seeds increases the total content of the phenolic compounds extracted with the oil from the seeds. Higher amounts of p-coumaric acid ethyl ester, vanillin, feruladehyde, *p*-coumaric acid, and syringaldehyde were found in the oil after the roasting process.

Chouigui et al. (2013) analyzed prickly pear seeds of different cultivars, they reported that the total flavonoid contents varied from 1.55 to 2.64 mg QE/100 g of DW and the total tannins from 4.1 to 6.6 mg CE/100 g of DW. These differences with our results could be attributed to different cultivar, geographical origin of the fruits, degree of maturity, the storage conditions and also to the extraction protocols and analytic assays.

3.4. Antioxidant activity

Antioxidant activity is one of the major mechanisms by which herbs and fruits deliver health benefits. The high amounts of polyphenols contribute to their ability to scavenge free radicals and to chelate metal ions involved in their production, which imparts strong antioxidant activity to prickly pear seeds (Chougui et al., 2013; Ghazi et al., 2015).

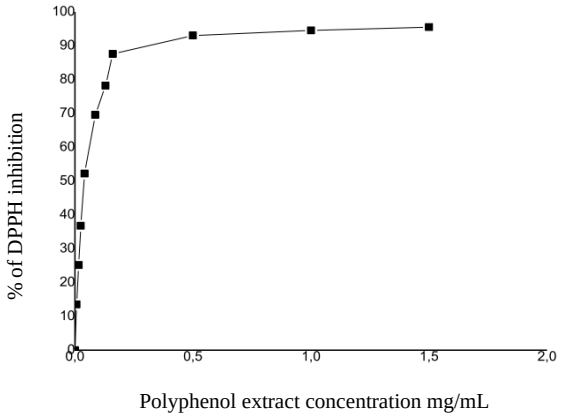
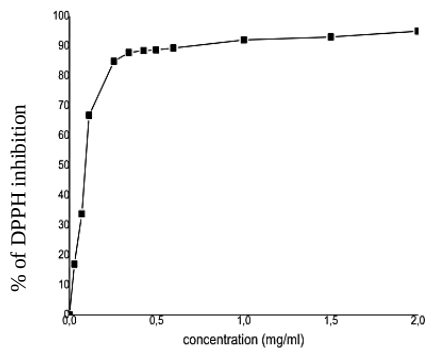


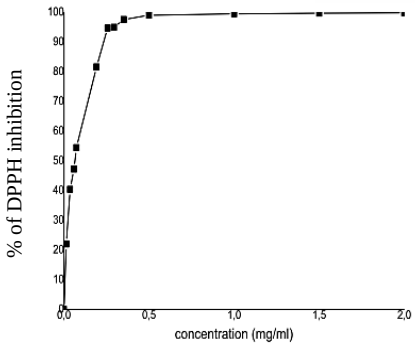
Figure 1. DPPH radical scavenging capacity of phenolic extract of OFI seeds.

An increase in percentage reduction in DPPH proportional to the concentrations of phenolic compounds extracts of prickly pear seeds as shown in (Figure 1). Effectivey, the inhibition of this radical reaches its maximum value (87.6% at a concentration of 0.16 mg/mL) then stabilizes and reaches 95% at the concentration of 1.5 mg/mL. We can deduce that polyphenols of prickly pear seeds are natural antioxidants that have capacities of fairly strong neutralization of DPPH compared to that of vitamin C, since the EC 50 of extracts of total polyphenols is 0.035 (mg/mL) and the EC 50 of vitamin C is 0.06 (mg/mL).

These results are in adequacy with de Wit et al., (2019) who showed that seeds were the tissue type with the highest antioxidant potential in comparison to cladodes, fruits pulp and fruits peel. Similarly, Chougui et al., (2013) focused research on antioxidants in the seeds of OFI, which were shown to be rich in polyphenols, flavonoids, and tannins, with higher concentrations of those molecules than in the fruit pulp.



flavonoid extract concentration mg/mL (ethyl acetate)



flavonoid extract concentration mg/mL (n-butanol)

Figure 2. DPPH radical scavenging capacity of flavonoid extracts of OFI seeds.

Regular consumption of flavonoids is associated with reduced risk of several chronic diseases and helps to protect the body. In addition, they have antioxidant, antiviral and antibacterial properties (Kozłowska and Szostak-Wegierek, 2014).

The EC_{50} of the flavonoid extract is clearly close to the EC_{50} of the ascorbic acid and particularly the EC_{50} of the n-butanol phase which is slightly higher than the EC_{50} of the acetate phase, as shown in (Figure 2). However, Dib et al., (2021) found that tannins expressed the most important inhibition of DPPH radical activity (IC_{50}= 10±0.008 µg/mL) than the other fracrtions and standard antioxidants as ascorbic acid and Trolox.

4. Conclusion

This work showed that the seeds of *Opuntia ficus indica* grown in Algeria had an intriguing nutritional value and possessed an appreciable amount of oil. The seeds also contained also important levels of phenols, flavonoid and tannin which contribute to the free radical scavenging power. It is advised to appreciate OFI seeds for their positive qualities in nutrition and cosmetics.

Conflict of Interest

The authors declare that they have no conflict of interest.

Author Contribution Statement

Meriem Belarbi designed the overall research experiments. Fatima Zahra Ghanemi and Abdelhafid Nani performed the experiments and data analysis. Meriem Belarbi, Fatima Zahra Ghanemi, Zoubida Mami and Darine Khaldi analyzed and interpreted the results. Fatima Zahra Ghanemi and Danish Patoli wrote and edited the manuscript.

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