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Original Article

# High Performance Liquid Chromatography of antioxidant activity of seeds some varieties of prickly pear (*Opuntia ficus-indica L.*) from the Sidi-Fredj Souk Ahras Algeria

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**ABSTRACT**

The study was conducted on seeds of three varieties of *Opuntia ficus-indica* (*O. ficus-indica*), harvested from different regions of Souk Ahras in north-East of Algeria (Sidi-Fredj, Taoura and Drea). The analysis consisted of antioxidants extraction, following the solid-liquid extraction with ethanol 40% (v/v), their quantification and determination of their antioxidant activity by two methods (reducing power and DPPH (2-2 diphenyl 1-picryl hydrazyl) Test). Results show that total phenolic content (TPC) ranged from 71,01to 90,79mg GAE (Gallic Acid Equivalent)/100g Extract. The Sidi-Fredj variety has the best rate followed by the Taoura and Drea ones. Concerning, flavonoids and tannins, the Taoura variety contains higher concentrations corresponding to 2.67 mg QE (Quercetin equivalent)/100g and 6.60mg CE (Cyanidin equivalent)/100g. Activities of extracts of three varieties show similar performance; the EC50 for the reduction of ferric iron 0.05g/mL for all extracts, whereas the EA is more important in Sidi-Fredj and Drea seeds extracts. Regarding the scavenging of DPPH, Drea and Sidi-Fredj varieties show the highest capacity equals to 0.20g/mL. Linear correlations between the different studied activities and some antioxidants (flavonoids, tannins) rates were noticed, indicating their participation in the obtained effect.
Analysis of the oil by HPLC revealed the presence of phenolic compounds including gallic acid and a form of vitamin E (α-tocophérol), capable of expressing biological activities; Anti-oxidants and vitamins. GC (Gas Chromatography) analysis showed that prickly pear oil was a major source of essential fatty acids (C18:2 and C18:3). The anti-free radical activity of the seed was evaluated through a DPPH chemical test. The results obtained show a hight reducing activity of our extract.

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## 1. Introduction

Medicinal plants are an inexhaustible source of bioactive natural substances. Indeed, plants secondary metabolites have the advantage of being a wide variety of chemical structures that make them apply in various medical, pharmaceutical, cosmetic, and agri-food fields [9]. *Opuntia ficu-indica* commonly called, barbarism fig, is native to the Mexico, well adapted to the climate of the Mediterranean basin [1]. This plant, has long been marginalized but its culture is growing, given its socio-economic, and environmental importance [2]. Indeed, modern medical research is rediscovering the plant and its properties with increasing interest. It studies the active molecules that make it up and enable it to fight effectively against some of the most serious diseases of our time such as arteriosclerosis, cholesterol and diabetes [3,4]. Despite its abundance especially in the region of souk Ahras, the fig of barbarism generates little interest; it is not valued and its consumption remains seasonal. However their importance continues to grow in other countries such as Mexico, Argentina, Spain and even neighboring countries (Morocco and Tunisia). The richness of their oil in unsaponifiable materials and fatty acids essential have in fact a good asset for its operation in cosmetology and its consumption as table oil [5,6]. In addition, the seeds contain various

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phytochemicals such as secondary metabolites (polyphenol, flavonoids and tannis) which could justify their industrial exploitation as a natural antioxidant [7, 8,9]. Too few studies have been reported by the literature on its phenolic composition as well as on its antioxidant activity. In Algeria, very little information exists on whether the fruit or the seed. The present study is part of the exploitation of the seeds of figs of local origin and aims to of phenolic compounds' composition and their antioxidant activity.

During this work, several aspects related to this topic were studied. The first aspect concerned the physico-chemical analyses carried out on the pulp of the fruit.

The second aspect concerned the physico-chemical characterisation of the extracted oil by carrying out analyses on HPLC [22]. The main focus was on the study of the saponifiable fraction (fatty acid composition), the unsaponifiable fraction (qualitative and quantitative determination of the main compounds) contained in this oil and the study of biological activity by testing the antioxidant activity.

## 2. Materials and Methods

### *2.1. Plant material preparation and study area*

The study was carried out on the seeds of three varieties of barbarism fig harvested in August 2021 from different regions of Souk Ahras regions (Sidi-Fredj, Ouled Mimoune, Taoura, Drea and Ouilene 45 km north of Souk Ahras) and selected according to the presence or absence of thorns, the color (the seeds green (Drea), yellow (Sidi-Fredj) and orange (Taoura)) and shape of the fruit. The barbarism fig were rinsed thoroughly with running water, then dried and peeled. The seeds were then separated from the pulp, rinsed and dried at room temperature for 24 h. The dried seeds were crushed using the electric grinder A11 basic (IKA, Germany) until a fine powder was obtained. It has been stored in jars protected from light and moisture[9].

#### *2.1.1. Colors associated to the different varieties*

Three varieties of cactus pear fruits were selected, yellow, orange and green Opuntia ficus-indica L. Mill, which were provided by the Cooperative of producers of Cactus and its derivatives (NOPALTEC) of Sidi-Fredj (Souk Ahras, North-East of Algeria). during September 2020 and were selected in agreement with the NOPALTEC legislation for non-industrialized food products relating to the human use of cactus pear (*Opuntia spp.*) fruit, being of firm consistency, clean, free of foreign matter, and free of damage caused by pests or diseases in addition to presenting a state of commercial maturity, determined by the observation of the sinking of the fruit receptacle [25]. The fruits were divided into three batches, washed, and manually peeled; the residues (mesocarp and pericarp), Three varieties grinding, as presented :

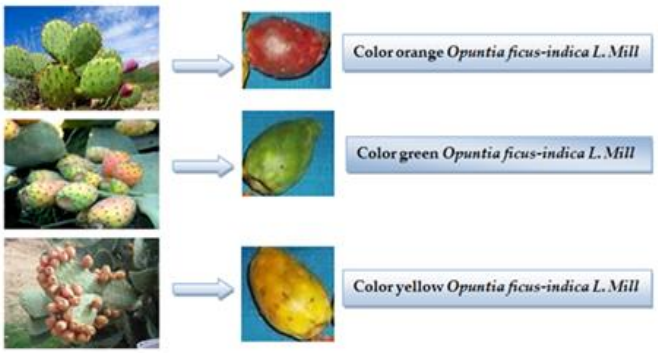


**Fig 1**. Colored ecotypes of prickly pears (Opuntia ficus-indica) (garden variety, Experimental Station of Sidi-Fredj (Souk Ahras, North-East of Algeria).

### *2.2. Analysis*

#### *2.2.1. HPLC Analysis*

Identification of barbarism fig seeds extract by Agilent model 1260 HPLC with DAD (Detector with diode) detector array, the principle is based on the comparison of the retention time of the analyte peak in the sample of barbarism fig seed oil with that of the pure stallion [11, 22].

High Performance Liquid Chromatography (HPLC) is separative adsorption or sharing where a mobile phase consisting of a mixture of solvents, buffered or not, of force variable ionic, passes through a column containing a stationary phase consisting of the microspherical particles with diameters between 2 and 5 micrometers or materials porous monotith. It corresponds to the technique of analysis and characterization of extracts in the most widely used phenolic compounds because it has a high resolution, high reproducibility, and a relatively short analysis coma It can be used for separation, quantitative determination, and identification of polyphenols, generally used in the reverse phase, and it would have three essential points: the column, elution solvent and detector. In the separation of a mixture, this chromatography involves two variables, the phase (column), and the mobile phase (the solvent or solvents).

HPLC analysis was performed on a Perkin Elmar Flexar system with a binary pump distribution, internal degasser, diode matrix detector (PDA) and Eclipse ODS Hypersil C18 column (15 cm 4.6 µm). The mobile phase consisted of a solvent A (water / formic acid) (0.1%), and solvent B (acetonitrile / formic acid) (0.1%). The elution system in gradient was: 95% A (5 min), 90% A (10 min), 50% A (35 min), 95% A (10 min) and 95% A (5 min). The flow rate is 1 mL/ min, and the volume of injection is equal to 20 µl. Computer software allowis a view of the signals recorded by the detector[22].

The measurements were carried out according to the following method with some modifications to the length d wave 280 nm [22].

The eluents were degassed; the extracts and standards were filtered over the millipore membrane to avoid damage to the column and to limit interference due to impurities.

Peak identification of phenolic compounds was based on their retention time and their comparison with the standards, the solutions of the standards are prepared by dissolving 10mg in 1 mL of methanol.

### *2.2.2. Identification and quantification by HPLC/ DAD*

The separation of phenolic compounds by HPLC was carried out by Agilent model 1260 with DAD detector. Quantification was performed by external calibration using standards. The chromatograms were saved and their treatments were exploited using Chem Station software (Agilent, Germany). Phenolic compounds are identified by comparing the peaks found to well-determined references from their wavelengths and retention times. The peak of each molecule is integrated and its surface is thus noted taking into account the standard solutions that allowed us to calibrate the HPLC device. The selected for calibration are analyzed at their maximum absorption wavelength. Each range has different concentrations that will be analyzed in HPLC to obtain a calibration line. The analysis was conducted under the following conditions:

Stationary phase C18 (Length 15cm)

Mobile phase: binary system

A: Water distilled at 2% acetic acid. B: methanol.

Programming the gradient:

0 min: 95% (a); 5% (B).15 min: 75% (a); 25% (B).20 min: 60% (a); 40% (B).24 min: 50% (a); 50% (B).

The 25 à 50 min 0% (a); 100% (B). Flow rate: 0.8 ml/min.

Injection loop: 20 µl. wavelength = 280 nm. Detector: DAD [22].

HPLC analysis was performed on Perkin Elmar Flexar system equipped with a binary pump distribution system, an internal degasser, diode matrix detector (PDA) and an Eclipse ODS Hypersil C18 column (15 cm 4.6 µm). The mobile phase consisted of solvent A (water/ formic acid) (0.1%), and solvent B (acetonitrile/ formic acid) (0.1%). The gradient elution system was 95% A (5 min), 90% A (10 min), 50% A (35 min), 95% A (10 min) and 95% A (5 min). The flow rate is 1 mL/min and the injection volume is 20 µL. Computer software to view the signals recorded by the detector. The eluents were degassed; the extracts and standards were filtered over the millipore membrane to avoid damage to the column and to limit interference due to impurities. The identification of the peaks of the phenolic compounds was carried out according to their retention time and their comparison with the standards, the solutions of the standards are prepared by dissolving 10mg in 1mL of methanol[22].

### *2.3. Moisture test*

The moisture content in the seed powder of each variety was determined according to the drying process in the oven; it consists of drying the powder at 105°C up to a stable weight. The moisture content (MC) was calculated by the following formula [9].

$$\mathbf{MC\ (\%) = (Wf - Wd) / Pf \times 100}$$

$\mathbf{W_f}$**:** weight of fresh sample (g)

$\mathbf{W_d}$: dry sample weight(g)

### *2.4. Extraction and determination of phenolic compounds*

The extraction of phenolic compounds was carried out according to the protocol, [10] 2 g of oil are extracted three times with 10 mL of a solution of methanol/water (80/20), after stirring (2 min) and centrifuging (5000 rpm for 30 min) to each extraction. Methanol extracts are collected and evaporated using rotary evaporator at reduced pressure, then taken up by 2 mL of acetonitrile and extracted three times per hexane (1mL). The acetonitrile fraction is dried, the dry residue is taken up by 2 ml of methanol. This extract is used for colorimetric determination, chromatographic analysis and determination of the antioxidant power of phenolic compounds [9, 22]. .

### *2.4.1. Determinations of Total Phenol Compounds*

For this experiment, Folin-Ciocalteu reagent was used, consisting of a mixture of phosphotungstic acid ($H_3PW_{12}O_{40}$) and phosphomolybdic acid ($H_3PMo_{12}O_{40}$), is reduced in the presence of phenolic compounds into a mixture of blue tungsten oxides and molybdenum. The coloring is proportional to the number of polyphenols present in plant extracts [11].

The content of phenolic compounds was estimated according to Ghasemzadeh *et al*. [12]. It consists of mixing 250µL extract from barbarism figs seeds and 1.5mL of Folin-Ciocalteu reagent. After 5min, 1.5mL of sodium carbonate $Na_2CO_3$ (6%) were summed. Absorbance was measured at 760nm after 1h of incubation in the dark. The concentration of phenolic compounds is determined using a curve calibration performed with different concentrations of gallic acid. The results are expressed as mg equivalent of gallic acid/100mg dry Extract (EGA/100g) [22]. All tests were conducted in triplicate.

### *2.4.2. Determination of flavonoids*

The flavonoid content was determined on the basis of the formation of a complex flavonoids-aluminum that absorbs at 430 nm. According to protocol described by Djeridane *et al*. [13]. The seed extract was added to 1.5mLof aluminum trichloride ($AlCl_3$: 2%). After incubation for 30 min at room temperature, the absorbance of the mixture reaction was read at 430 nm. The content of flavonoids in extracts was calculated by reference to a curve calibration with quercetin. Results are expressed in mg quercetin equivalent /100mg dry Extract (mg EQ/100g).

### *2.4.3. Determination of tannins*

The condensed tannins were measured using the Butanol-HCl method, developed by Iqbal *et al*. (2011) [14], based on a tannin depolymerisation reaction condensed in an acid medium. This reaction leads to the

release of anthocyanidins (coloured molecules) corresponding to the cleaved monomers. The reaction medium, consisting of a known amount 5g of each seeds powder extract dissolved in 25 mL reagent [n-butanol: HCl, 3:2 (v:v) and 0.385 mg iron ammonium sulphate], was placed in the oven at 95°C for 15 min. Afterwards, the absorbance of the same solutions was measured at 530 nm, and the results were determined by the following formula[9].

$$\mathbf{TC = A \times DF \times MC \times 1000/\varepsilon}$$

**TC** : tannin concentration in mg/L;A : absorbance recorded at 530 nm.

**DF** : dilution factor.

**MC** : the molar mass of cyanidine (287.24 g/mol).

**ε:** the molar extinction coefficient (34700 L/mol).

### *2.5. Antioxidant activities*

#### *2.5.1. Reducing power*

The power to potassium ferrocyanide is the ability to ferrous iron ($Fe^{+2}$) extracts in the presence of antioxidants in iron ($Fe^{+3}$) [15, 16]. The reducing power of barbarism fig extracts has been determined according to the Nagulendran *et al.* (2007) [17]. Method with some modifications. Different concentrations of each extract were first prepared (ranging from 0.027 to 0.1 g/mL). 0.5mLof each concentration was mixed with 1.25mLphosphate buffer (0.2M and pH 6.6) and 1.25mL potassium ferricyanide [$k_3Fe(CN)_6$] at 1%. After 20 min incubation at 50°C, the reaction was stopped by adding 1.25 ml of trichloracetic acid (10% TCA). 1.25mLof the mixture was taken and added to 1.25ml of distilled water and 0.25mL of chloride iron ($FeCl_3$) at 0.1%. After incubation for 30 min, absorbance was measured at 700nm against calibration curve with ascorbic acid at different concentrations were used to quantify the reducing power of phenolic compounds in extracts. Results are expressed in mg ascorbic acid equivalent/100mg of Extract (mg AAE/100g) [9].

#### *2.5.2. DPPH Antiradicalar activity*

The method uses the radical DPPH (2-2 diphenyl 1-picryl hydrazyl). The activity of the DPPH radical was estimated according to the protocol described by Lopes-Lutz et al. (2008) [18]. Different extract concentrations were first prepared (ranging from 0.08 to 0.33 g/ml). Each test solution was mixed with 2.44 mL of solution DPPH (6x10-5 M). Samples were placed in darkness and at room temperature for 1h. The absorbance reading was made at 517nm against a white. The anti-radical activity, which expresses the ability to trap free radicals, has been estimated by the percentage inhibition of DPPH according to the following formula [9].

$$\mathbf{I\% = A_C - A_E / A_C \times 100}$$

**I%**: percentage inhibition; $\mathbf{A_C}$**:** absorbance of the DPPH solution; $\mathbf{A_E}$: the absorbance of the sample.

The percentage inhibition is used to calculate the $EC_{50}$values, defined as the effective concentration of the extract required to reduce the concentration of initial DPPH. The $EC_{50}$ are determined graphically by the regression line abscissa represents the concentration of the samples and the ordered the antiradical activity in percentage. The antioxidant capacity of a compound is all the higher as its $EC_{50}$ is small [20, 21, 22]. Another parameter that is often determined, in addition to the $EC_{50}$, is inversely proportional to the $EC_{50}$ ($EA = 1/EC_{50}$) [22]. .

#### *2.5.3.* Statistical analysis

The results obtained were analyzed using the STATISTICA 5.5 software for analysis of variance with a single classification criterion (ANOVA), whose degree of significance of the data is taken at the probability of $P \leq 0.05$. Results with different letters are significantly different (a>b).

## 3. Results and Discussion

### 3.1. HPLC Analysis Results

Identification of barbarism fig seeds extract by Agilent model 1260 HPLC with DAD (Detector with diode) detector array, the principle is based on the comparison of the retention time of the analyte peak in the sample of prickly pear seed oil with that of the pure stallion [11, 22].

The principle of the optimized method consists in dissolving 0.2 g of oil in 4mLof hexane; the detection is done by an HPLC/DAD. 10 mg of the standard is dissolved in 50 mL of hexane, starting from this stock solution further dilution has been prepared to plot the calibration curve. After this preparation, A volume of 20 µL will be taken from the solution and injected into the HPLC under the conditions following: The 25 cm long steel column; Stationary phase C18; Mobile phase: (75% methanol; 25% acetonitril); Flow rate: 1.5 mL / min; Wavelength: 280 nm; Scan time: 15 min. in order to compare their chromatographic profiles with those of the standards and to obtain information on the chemical nature of the constituents, the mobile phase used to qualitatively analyze our extracts is a mixture of solvents: formic acid and acetonitrile. Fifteen pure phenolic compounds were used in the HPLC analysis as standards, their retention times (RT) represented in the Table 1.

Table 1. Rétention time for the standards used [22, 21].

| Standard | Retention time in (min) |
|---|---|
| Chlorogenic acid | 1.514 |
| Gallic acid | 2.265 |
| Hydroquinone | 3.717 |
| Resorcinol | 3.726 |
| Pyrocatechol | 6.487 |
| Catechin | 15.918 |
| Hydrated catechin | 16.735 |
| Syringic acid | 18.675 |

| | |
|---|---|
| Caffeic acid | 19.174 |
| P-coumaric acid | 24.791 |
| Ferulic acid | 26.483 |
| Sinapic acid | 26.297 |
| Rutin | 28.171 |
| Naringenin | 31.531 |
| Quercetin | 39.394 |

Results of HPLC analysis of *Opuntia ficus-indica* seed extracts are represented by the retention times [22, 09].

The constituents contained in each extract analyzed were identified by HPLC chromatogram (Figure B, C et D) with chromatogram of standard compounds (Figure A) based on the retention times obtained under the same conditions. Analysis of the chromatogram obtained shows that the hydroalcoholic extract (Extract Ethanol 40%) contains all the phenolic compounds with fourteen peaks except for the hydrated catechin, while two compounds (Hydroquinone, Catechin) for the crude methanol extract (Crude Methanol), and three compounds in the methanol extract (aqueous acetone) (Hydroquinone, Pyrocatechol, Catechin hydrate), which were not identified Table 2.

Meaning that our extracts are rich in polyphenols (phenolic acids) about to with concerning flavonoids, the areas of the peaks obtained show that the quantities differ from one extract to another, the whose highest rate of chemical substances is represented by the hydroethanolic extract, and this reflects, and confirms our results of the specctrophotometric assay with total phenol levels for this extract (144.5 mg EGA(Equivalent in Gallic Acid) /100 g DM(Dry Matter)) Versus flavonoids (44 mg EQ (Equivalent in quercetin)/100 g DM (Dry Matter)) [22].

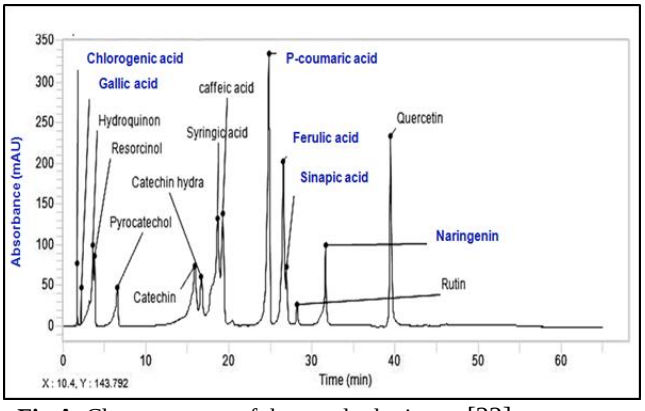


**Fig A.** Chromatogram of the standard mixture [22].

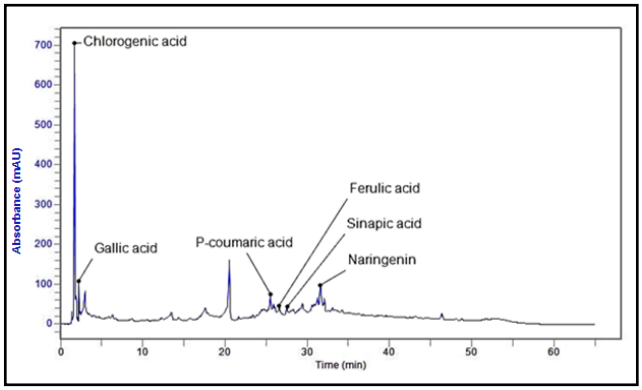


**Fig B**. HPLC chromatograms of *O.ficus-indica* seed extracts (Extract Ethanol 40%) at 280 nm [22].

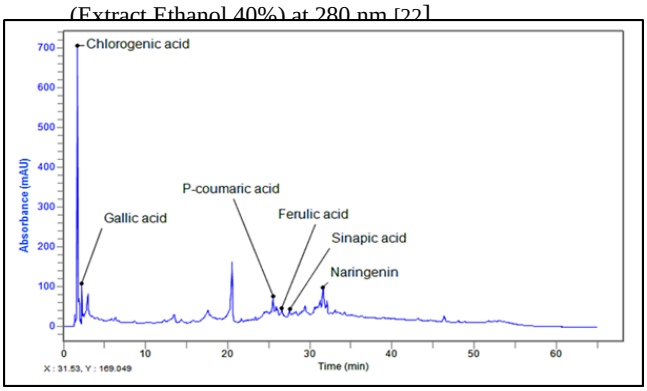


**Fig C.** HPLC chromatograms of *O.ficus-indica* seed extracts (Crude Methanol) at 280 nm [22].

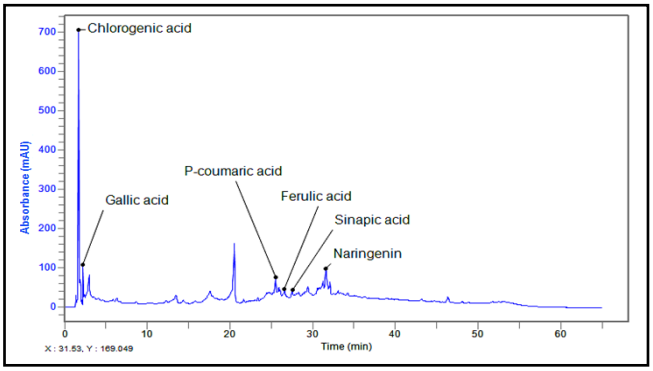


**Fig D.** HPLC chromatograms of extracts of *O.ficus-indica* seed (Aqueous acetone) at 280 nm [22].

Table 2. Identification of different constituents of *Opuntia ficus-indica* seed extracts different regions (Sidi-Fredj, Taoura and Drea) of Souk Ahras North-East Algeria).

| **Extracts / Standards** | **Extract Methanol 40%** | **Crude Methanol 100%** | **Fractionated methanol** |
|---|---|---|---|
| Chlorogenic acid | +T | + T | + SF |
| Gallic acid | +D T | + SF | + SF |
| Hydroquinone | + SF | -D | -T |
| Resorcinol | +D | +D | +D |
| Pyrocatechol | + SF | + SF | - SF |
| Catechin | + SF | -D | + SF |
| Hydrated catechin | -T | + SF | -D |
| Syringic acid | + SF | + SF | + SF |
| Caffeic acid | + SF | + SF | + SF |
| P-coumaric acid | + D | + SF | + SF |
| Ferulic acid | + D | + D | + D |
| Sinapic acid | + SF | + SF | + SF |
| Rutin | + SF | + SF | + T SF |
| Naringenin | + SF | + SF | + SF |
| Quercetin | + **T** | + T | + T |

**SF:** Sidi-Fredj; **T**: Taoura; **D**: Drea.

Our results showed that the vast majority of bioactive compounds of Opuntia are soluble in water, that is to say, that to obtain fractions rich in polyphenols, it is preferable to use a mixtures of the appropriate organic solvent with water, like ethanol, whose maceration with 50% ethanol at room temperature for 24 hours is the perfect technique for

extracting the hydroethanolic extract (Extract Ethanol 40%) which combines both a high yield, a polyphenol content, and significant antioxidant activity. The results of the HPLC analysis confirm those of the colorimetric analysis through the identification of some phenolic compounds. Our results also showed that qercetin, and Routine were most commonly found in the various extracts of our plant; this would be consistent with the results of [22, 23].

These data suggested that the seeds of the cactus "*Opuntia ficu-indica*" could be a potential source of a natural compound and reveal that the polar extracts in particular (hydro-alcoholic) of this species are promising sources, and therapeutic agents for the search for new natural active ingredients.

### 3.2. Determination of antioxidants

There are different antioxidant components in prickly pear seeds such as flavonoids and tannins that account for more than half of phenolic compounds [8, 22, 9]. However, our interest has focused more on their composition and oil content, and very little work has been done on their composition and antioxidant activity, making it difficult to compare.

The results of the determinations of all the antioxidants (total phenolic compounds, flavonoids, tannins) in the seed extracts of the three varieties of prickly pears, determined spectrophotometrically according to several processes, are summarized in the following table 3.

Table 3. Contents of total phenolic compounds, flavonoids, tannins of three varieties of barbarism fig (different regions of Souk Ahras North-East).

| Sample | Compounds total phenolic(mg GAE (Gallic Acid Equivalent)/100g) | Flavonoids (mgEQ/100g) | Tannins (mgEC/100g) |
|---|---|---|---|
| **Taoura** | 76,04±1,29[b] | 2,67±0,54 [a] | 6,60±0,09 [a] |
| **Sidi-Fredj** | 90,79±0,89[a] | 1,50±0,08 [b] | 4,70±0,07 [b] |
| **Drea** | 71,01±1,75[b] | 1,50±0,07 [b] | 4,54±0,33 [b] |

Values with the same letters do not differ significantly. a>b.

#### 3.2.1. Total phenolic compounds (TCP)

The TCP were determined spectrophometrically by the Folin Ciocalt method; based on phenolates oxidation and polyheterocyclic reduction, followed by the formation of the blue molybdenum-tungsten complex which propotionally to the concentration in these compounds [23, 24]. Results of the contents of the ethanolic extracts of the seeds of the three varieties of figs of TPC (Total phenolic compounds) are presented in Table 3. Concentrations range from 71.01mg 90.79 mg EGA /100g. The highest value was recorded in the extract of the variety yellow followed, at the same level, by extracts of the orange and green varieties compared with the seeds of other species Concentrations range from 74.07mg 92.80 mg EGA /100g [9]. The highest value was recorded in the extract of the variety yellow followed, at the same level, by extracts of the orange and green varieties.In The study a variety of effects at the $p \leq 0.05$ threshold. However, only one difference between the TPC content of the yellow variety and that of the other varieties which seem to be very close. Levels reported by[8] for the same species, in Mexico City cultivars, Montesa (yellow-brown), Cristalina (light green), and Pelón-liso (red-violet), are higher than the results obtained in this study and they range from 337 to 460 mg EGA /100g DM (Dry Matter) [9]. This difference can be attributed to either methods of extraction and analysis, the geographical origin of the sample, degree of maturity, or storage conditions. Indeed, the seeds used in this study derive fruits that were harvested in August 2021 and it is likely that during their storage, the degradation of some compounds. Compared with the seeds of other species, these contents obtained in the seeds of figs of barbarism are superior, for example, to those in the seeds of some varieties of d'*O. ficus-indica* that are between 16.66 and 31mg EGA /100g DM (Dry Matter) [27, 9].

#### 3.2.2. Flavonoids

Flavonoids have a free hydroxyl group in position 5, capable of giving the presence of aluminum trichloride a yellowish complex, by ion chelation aluminum ($Al^{3+}$), which is proportional to the concentration of flavonoids present in the excerpt [22,14,9]. Results of the determination of flavonoids obtained for the seeds of the three varieties studies of d'*O. ficus-indica* (Table 3) show that the concentration is significantly higher in the seed extract of the orange variety; it is 2.67 mg EQ/100g versus 1.50 mg for green and yellow variety extracts compared it is 2.64 mg EQ/100g versus 1.55 mg for green and yellow variety extracts These values are also lower than those reported by Cardador-Martínez *et al.* [8]. and which are between 46 and 50 mg ECa/100g DM (Dry Matter) [9]. The same observation was noted with the contents seeds of other species such as tomatoes (10.33-14,65mg ERu/100g) [27, 9]. It should be noted that the flavonoid content is often expressed in different standards equivalents (qurcetin, rutin, catechin) and the nature of the standard used could therefore, in more factors, mentioned above, influence the final result.

#### 3.2.2. Tannins

The astringency of fruits and drinks often stems from the interaction of polyphenols with salivary proteins [29]. The n-butanol method is colorimetric method consisting of a Proanthocyanidins oxidizing cleavage with ferrous sulfate to allow the Determination of condensed tannin content [25, 9]. Tannin content of seed samples of the varieties studied Table 3. have concentrations ranging from 4.54 to 6.60mg CE/100g compared have concentrations ranging from 4.50 to 6.6mg CE/100g[9]. Seeds of the variety orange are, once again, the richest extract followed by that of the yellow variety with a value equal to 4.79mg which is not, statically, different from that of the various green. These contents are also very low compared to those recorded by [8, 9]. with an interval between 137 and 205mg CEa/100g DM (dry matter). The type of cultivar and the above factors may be related to these differences.

### 3.3. Antioxidant and antiradicalar activities

#### 3.3.1. The reducing power (RP)

The assay measures the reduction of ferric ion ($Fe^{3+}$)-ligand complex to the intensely blue-coloured ferrous ($Fe^{2+}$) complex by antioxidants in an acidic medium [30, 31]. Many authors consider the reductive capacity of a compound as a significant indicator of antioxidant potency [16, 23, 30, 9,10].

The reduction causes the color change, there is a turn of the yellow color of the potassium ferricyanide in green blue whose intensity depends on the reducing power of each extract. The increase in absorbance indicates an increase in reducing power [32, 9]. The results of the evaluation of the reducing power of the seed extracts of the three varieties of *O. ficus-indica* studied, are illustrated by the following figure. According to the results obtained, the reducing power increases with the increase in concentration of seed extracts from all three varieties Figure 2.

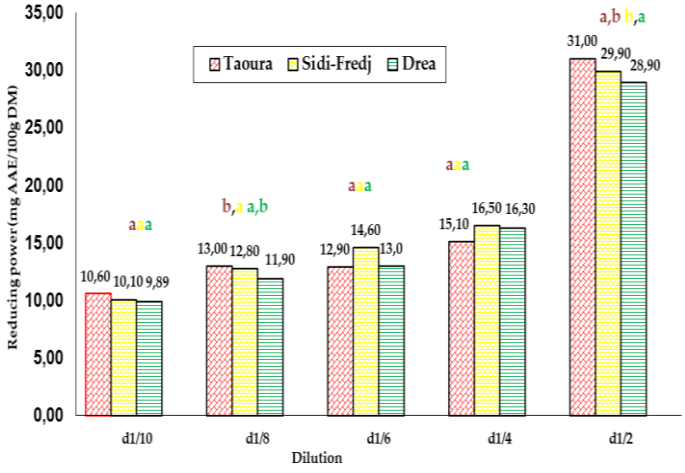


Fig 2. Reducing power of the seed extracts of the three varieties of prickly pear (different regions of Souk Ahras North-East).Values with the same letters have no significant differences (a>b). The vertical bars represent the deviations.

This observation of reducing power $CE_{50}$ and EA was confirmed by several authors [18, 32, 33]. The plotted curves evolve in the same way and the values obtained oscillate between 9.89 and 31mg AAE (Ascorbic acid equivalent) /100g for all samples. Over all the concentrations tested, significant differences were noted only at dilutions D1/8 and D1/2 and it appears that, the extract of the seeds of the various green (Drea) is the one with the best reducing potential. From the different measurements, the parameters $CE_{50}$ and AE were deduced for each sample (Table 4). The $CE_{50}$ was the same for all seed extracts and is equal to 0.049 g/ml, while the AE varies by variety, from 19.00 to 19.45 compared the same for all seed extracts and is equal to 0.05 g/ml, while the AE varies by variety, from 19.02 to 19.99[9]. the most effective are represented by the green (Drea) and yellow (Sidi-Fredj) varieties. As shown in Table 4, the $EC_{50}$ required to reduce the DPPH by 50% is 0.26 g/ml for the seed extracts of the two orange and green varieties; for the variety yellow, it took a higher amount (0.3g/ml) [9]. The AEs correspond oscillate between 3.31 and 3.91 and confirm the same order [9].

Table 4. Reducing power ($CE_{50}$ and EA) of the seed extracts of the three varieties of barbarism fig (different regions of Souk Ahras North-East).

| Sample | $CE_{50}$ (g/ml) | AE (antiradicalar efficacy) |
|---|---|---|
| ***Taoura*** | 0,059±0,003[a] | 19,00±0,131[b] |
| ***Sidi-Fredj*** | 0,047±0,007[a] | 18,221±0,358[a,b] |
| ***Drea*** | 0,049±0,001[a] | 19,457±0,049[a] |

Values with the same letters do not differ significantly. a>b.

#### 3.3.2. Activity by DPPH

This test is not quantitative, it allows to compare different extracts between them according to their ability to trap the DPPH radical and thus appreciate the qualitative variations of phenolic compounds. The antiradicalar activity of *O. ficus-indica* extracts was tested using a Free radical stable methanolic solution 2,2-diphenyl-1-picrylhydrazyl (DPPH) [35, 36] compared quercetin used as standard. The DPPH method is based on the ability of compounds to act as radical trappers by giving a hydrogen atom [36]. Results of the evaluation of the antiradical activity of the three seed extracts varieties of *O. ficus indica* studied, are illustrated by the Figure 3. Based on the results obtained, the anti-radical power of seed extracts from three varieties increases dose-dependent [32]. The percentage inhibition of the DPPH radical for the orange (Taoura), yellow(Sidi-Fredj) and green (Drea) varieties increase, for concentrations ranging from 0,071 to 0,5 g/ml, from 19.21 to 57.91%, 20.10 to 5% and 20.81 to 57.10% respectively with the predominance of orange (Taoura) and green (Drea) varieties Compared with the seeds of other species 0,075 to 0,3 g/ml, from 20,74 to 58,51%, 20,82 to 52,58% and 22.19 to 56.61% respectively with the predominance of orange and green varieties[9]. $CE_{50}$ and EA were also determined for each sample in order to free the DM elves of the different concentrations tested [9]. They are presented in the following Figure 3:

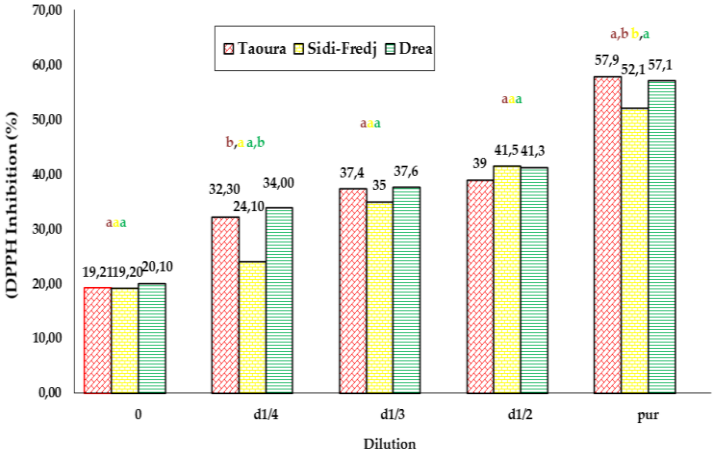


Fig 3. Percent inhibition of DPPH (Radical 2,2-diphenyl-1-picrylhydrazyl) from seed extracts of the three varieties of barbarism fig (different regions of Souk Ahras North-East).
Values with the same letters do not differ significantly a>b.

## 4. Conclusion

The results showed interesting levels of fig's antioxidants with an effective variety. The concentration of total phenolic compounds is higher in the yellow variety, flavonoid and tannin contents are more important in the variety orange. Antioxidant activity varies, too, depending on the variety; the green variety has better reducing power, and the orange variety has better anti-adical activity. Positive and negative correlations were observed between the different activities and the concentrations of antioxidants measured, this indicates that these compounds participate, according to the concentration, in the antioxidant and antioradical effects but, also, that the concentration factor alone does not explain the results obtained. Therefore, it would be desirable to purify and identify the compounds present in seed extracts, apply more experimental protocols developed, carry out the test on other varieties and several of samples more important by applying more elaborate experimental protocols. it would be interesting to complete this study with analyses on other parts of the fig tree that are considered again as worthless waste, test the effect of these bioactive agents in vitro, a key step in the search for biologically active natural source substances in order to predict their effect on human health. The seeds are a good source of high-quality oil but they also contain other bioactive compounds that will need to be considered and that could be exploited in different sectors.

The results obtained by HPLC showed that of *Opuntia ficus-indica* seed extracts oil exhibits a special quality by possessing traces of myristic fatty acid (C14) comprarant with varieties from other countries. It also contains amounts of α tocopherol and gallic acid (phenolic compounds) [22].

This particular composition would give it beneficial properties resulting in antioxidant effects. Antioxidant assays revealed the richness of the seed in total polyphenols. Therefore, the methanolic extract from the seed showed an interesting activity in trapping the DPPH radical. Despite these various uses, Opuntia ficus-indica remains insufficiently exploited. It contains potentialities until today remains unknown and whose updating could give this tree a new boom in its context.

Finally, seeds are a good source of high-quality oil, but they also contain other bioactive compounds that need to be considered and could be exploited in different sectors;

Furthermore, the introduction on the Algerian market of fresh juices and jams of barbarism fig will completely revolutionize the food industry, in terms of supply of pulp and seeds; It would be interesting to develop research lines in the direction of promoting this culture, developing the drying of pulp by atomization and making mixes for different food uses (juices, aromas, ice creams and sorbets).

## Appendix

Appendixes, if needed, appear before the acknowledgment.

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### Conflict of Interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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