Phytochemical Characterization and Antioxidant Activity of Cladodes of Opuntia Ficus Indica Cultivated in the Mascara Region (North-West of Algeria)

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Abstract

The Barbary fig (Opuntia ficus indica), a cactus belonging to the Opuntia genus, which holds multiple virtues, remains underutilized in Algeria. The cladodes of the Barbary fig are traditionally used as precious foods as well as in folk medicine. This study is designed to contribute to the valorization of Opuntia ficus indica paddles cultivated in Mascara (Northwest Algeria) by determining the phytochemical composition and evaluating antioxidant activity.The phytochemical characterization of the cladodes has shown that they represent significant sources of bioactive substances with interesting physicochemical properties. The antioxidant power results demonstrated that the methanolic extract of O.F.I cladodes is more active than other extracts across all 3 tests conducted (β-carotene bleaching, DPPH, and FRAP) with lower IC50 values (1,970µg/ml, 4,816 µg/ml, 3,98 µg/ml). Additionally, at the same tested concentrations, the inhibition percentages obtained by the DPPH and FRAP tests are lower than those of the β-carotene bleaching method.

Keywords: Barbary fig cladodes; phytochemical composition; antioxidant activity; methanolic extract.

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Introduction

Great interest has been given in recent decades to the expansion of Opuntia cultivation worldwide. This interest is motivated by its ecological role in combating desertification and soil degradation, and in creating conditions for agro-silvopastoral development. Numerous studies have been dedicated to its use as fodder, food, and for its role as a medicinal plant, additive in cosmetic and pharmaceutical products (Hadj Sadok, 2010).

The Barbary fig (cactus) is a plant native to arid and semi-arid regions of Mexico. It was introduced to North Africa around the 16th century. It is a robust plant that can grow up to five meters in height with a thick, woody trunk (Habibi, 2004). Its cultivation requires low investments and can

generate significant income. Moreover, ecologically, it is highly useful for erosion control and soil stability (Neffar, 2012).

In Algeria, Barbary fig (cactus) cultivation is carried out for traditional purposes such as hedge delineation, erosion control, or for fresh fruit consumption. Over the past three decades, several studies have been conducted on Algerian steppe and desert ecosystems with the aim of vegetation fixation and regeneration. Works focusing on the characterization and valorization of Barbary fig have also been initiated but often remain underexploited and scattered. Algeria possesses 80% of arid to semi-arid land, with strong points (climate, phylogenetic resources, and terrain) for developing Barbary fig cultivation. Opuntia plantation covers an area of 52,000 hectares in Algeria, with over forty varieties, six of which have edible fruit (Hadj Sadok, 2010).This species has shown good behavior and adaptation to environmental conditions, leading to increased soil organic matter and floristic richness. Algerian Barbary fig is not only renowned for its succulent and sweet taste but also contains a large quantity of seeds compared to those from Morocco or Tunisia. Some consider it among the best in the entire Mediterranean basin after Sicily (Cherif, 2016).

Opuntia, like other cacti, is regarded as a living water reserve that has enabled the survival of herds without water supply for several months. This is why the cactus has earned the name "miracle plant". The paddles are used as fodder for livestock, providing energy and water. Recently, these paddles have been used for water treatment. Their therapeutic benefits lie in their ability to reduce blood sugar and cholesterol levels, as well as their antioxidant properties due to their richness in fiber, vitamin C, and polyphenols (Schweizer, 1997).

In this study, we focused on examining a part of the Barbary fig: the cladode, which constitutes a by-product and could be exploited in various applications. The paddles are characterized by their health effects and richness in bioactive compounds. Among these, antioxidants and radical scavengers exert a protective effect in several diseases (Boutakiout, 2015).To highlight the Barbary fig, we focused on the paddles, which are still poorly known to the general public but widely consumed in Mexico as a vegetable. Studies on paddles are numerous; however, they have not yet been studied in the Mascara region. Hence, a study was initiated on Barbary fig cladodes in Northwestern Algeria (Mascara). Therefore, the objective of this research is to determine the physicochemical and phytochemical composition of cladodes cultivated in the Mascara region, as well as to analyze their antioxidant activity.

Materiel and Methods

Study Objective

Opuntia ficus-indica is a cactus native to Mexico introduced to North Africa in the 17th century by the Spaniards and primarily cultivated for its fruit production. The young shoots are also used in human consumption (nopalitos) in Mexico and in the southern United States (M.E. Malainine, 2001).

The objective of this study is to investigate the cladodes of Opuntia ficus-indica through the characterization of their phytochemical composition and the determination of the antioxidant power of various extracts from the cladodes of Opuntia ficus indica. Our work was conducted at

the laboratory of the Faculty of Science and Nature at the University of Mascara during the period spanning from February 28, 2022, to April 20, 2022.

Plant Material

The cladodes of Opuntia ficus-indica were collected from the region of Sidi Dahou in the Mascara province in February 2022.

I. Physicochemical Composition

I.1. Cladodes of the prickly pear cactus

The cladodes of Opuntia ficus-indica inermis (cladodes without spines), with masses ranging from 400 to 600 g, were washed with distilled water and longitudinally cut. Subsequently, the plant material was dried in a convection oven at 50°C for 6 hours. The dried products were ground in a spice grinder, sieved through a 250 µm sieve, and the obtained powders were stored away from light and in darkness. The drying yield was calculated according to the following formula and expressed as a percentage:

Drying Yield % = mass of powder / mass of fresh plant material × 100

Figure 01: Photos of the cladodes of the prickly pear cactus and their powders.

I.2. Moisture Content

The moisture content was determined by evaporating the water contained in the cladodes of the prickly pear cactus (PCF) placed in an oven at 100°C until a constant mass was obtained. The moisture content, expressed as a percentage, was calculated using the following formula:

Moisture Content % = mass of water / mass of plant powder × 100

Where:

Mass of water = mass of plant powder - mass of dry matter

I.3. Ash Content

The ash content was determined by ashing 1 g of dried sample for eight hours at 600°C (A.O.A.C., 1995). Each sample was placed in a pre-weighed porcelain crucible. The ashed samples were cooled

in a desiccator for 1 hour before being weighed on a balance with a precision of 0.1 mg. The ash content, expressed as a percentage, was calculated as follows:

Ash Content % = mass of ash / mass of dry matter × 100

I.4. Determination of Mineral Composition

I.4.1. Organic Matter Destruction

The mineral content determination was preceded by the complete elimination of organic matter present in the sample to be analyzed. Therefore, following the ashing of 1 g of dry matter, acid digestion was performed by adding 5 ml of 1 N nitric acid and 5 ml of 1 N hydrochloric acid. The mixture was then heated until white fumes appeared and the acids evaporated. After three washes with deionized water, evaporation was carried out. The mixture was cooled, and the volume was adjusted to 100 ml with deionized water.

I.4.2. Mineral Element Assay

The obtained solutions were analyzed by atomic emission to determine the mineral contents (K, Ca, Na) of the analyzed sample.

I.5. Lipid Content

The lipid content was determined using the Soxhlet method. The method involves exhausting the powder from the prickly pear cactus cladodes with hexane, which is then evaporated, leaving only the fat in the flask, which is quantified. The lipid content was calculated as follows:

Lipid Content % = mass of lipids / mass of dry matter × 100

I.6. Carbohydrate Assay

The composition of carbohydrate samples was determined using the method of Miller et al., (1959). From an initial solution with a concentration of 10 mg of sample/ml, a sample of 500 µl was taken, to which 500 µl of distilled water and 3 ml of 3,5-dinitrosalicylic acid (DNS) reagent were added, and the mixture was boiled in a water bath at 100°C for 10 min. Then, the volume was adjusted to 20 ml with distilled water, and the optical density of the obtained solution was measured at 550 nm against a blank containing water and DNS. A standard glucose range of 1 g/l was prepared to determine the content of reducing sugars, expressed in µg. The glucose content in the analyzed cladodes was expressed in mg/g of dry extract from the calibration curve.

I.7. Chlorophyll Assay

The total chlorophyll content was determined according to the method described by Mssadak (2018). 0.5 g of plant material was ground in 5 ml of 80% acetone using a mortar and pestle. After filtration through a Whatman No. 2 filter paper, the extract was adjusted to 15 ml with 80% acetone solution. The total chlorophyll content was determined by spectrophotometry as follows:

Total Chlorophyll µg/ml = 17.32 (DO645) + 7.18(DO663)

Where:

DO645: absorbance at 645 nm; DO663: absorbance at 663 nm.

II. Phytochemical Study

II.1. Extract Preparation

Four solvents (water, ethanol, methanol, and hexane) were used for extraction. A mass of 25 g of prickly pear cactus cladode powder was macerated with 300 ml of each solvent for 24 hours. Then, the solvent was evaporated using a rotary evaporator. Finally, the obtained extracts were kept in darkness at 4°C until further analysis.

II.2. Total Polyphenol Assay

In an alkaline medium, polyphenols reduce the Folin-Ciocalteu reagent to form tungsten and molybdenum oxide, resulting in a blue color. The intensity of this blue color indicates the total polyphenol content in the mixture. A sample of 125 µl of the 1 mg/ml concentration extract was mixed with 500 µl of distilled water and 125 µl of Folin-Ciocalteu reagent (a mixture of phosphotungstic acid and phosphomolybdic acid). After vigorous shaking and incubation for 6 minutes, 1250 µl of a 7% aqueous solution of Na2CO3 was added to the mixture, and the final volume was adjusted to 3 ml with distilled water (Dewanto et al., 2002). The mixture was incubated for 90 minutes at room temperature in the dark. Finally, absorbance was read at a wavelength of 760 nm.

A standard curve was prepared using gallic acid at concentrations ranging from 50 to 500 µg/ml. The total polyphenol content was expressed as milligrams of gallic acid equivalent (GAE) per gram of extract.

II.3. Flavonoid Assay

The quantification of flavonoids in the extracts was carried out using a colorimetric method (Dewanto et al., 2002). A 250 µl sample of the 1 mg/ml concentration extract was added to 75 µl of a 5% aqueous solution of NaNO2. After 6 minutes of incubation at room temperature, 150 µl of a fresh solution of aluminum chloride (AlCl3, 10%) was added to the mixture. After 5 minutes of incubation at room temperature, 500 µl of 1 M NaOH was added to the mixture. Then, the volume was adjusted by adding distilled water to 2.5 ml. After agitation, the absorbance of this preparation was measured at 510 nm with reference to a blank devoid of extract. A standard curve based on quercetin was also prepared at concentrations ranging from 20 to 220 µg/ml. The flavonoid contents were expressed as milligrams of quercetin equivalent (QE) per gram of extract.

III. Antioxidant Activity

Antioxidant activity was determined by three complementary methods:

III.1. Anti-radical Activity

The anti-radical activity of PCF extracts was measured according to the method described by Kirby and Schmidt (1997). This assay is based on the reduction of 2,2-diphenyl-1-picrylhydrazyl (DPPH•), a stable free radical with a dark violet color. After reduction by hydrogen from an antioxidant contained in the extract, this radical turns pale yellow. In the experiment, 500 µl of the

sample at the desired concentration (from 0.1 to 1 mg/ml) was mixed with 375 µl of 99.5% ethanol and 125 µl of a 0.02% DPPH• solution in 99.5% ethanol. A range of concentrations of the reference molecule ascorbic acid was prepared in the same way. The mixture was incubated in darkness for 60 minutes, and then the absorbance was measured at 517 nm. The anti-radical activity was calculated as follows:

DPPH• Inhibition % = (C - E) / C × 100

Where:

C: absorbance of the control (without extract);

E: absorbance of the solution containing the extract.

III.2. β-carotene Bleaching Test

This test was performed according to the method described by Koleva et al. (2002). An emulsion of β-carotene and linoleic acid was prepared as follows: 0.5 mg of β-carotene dissolved in 1 ml of chloroform was mixed with 25 µl of linoleic acid and 200 µl of Tween 40. The chloroform was completely evaporated using a rotary evaporator at 40°C. The residue from evaporation was then dissolved in 100 ml of distilled water. The obtained emulsion was freshly prepared before each test. A 500 µl aliquot of the ethanolic extract at various concentrations was introduced into tubes containing 2.5 ml of the solution prepared from β-carotene and linoleic acid. The mixtures were incubated for 2 hours at 50°C. The absorbance was measured at 470 nm before and after incubation. Controls and blank tests were prepared using the same procedure. The control for each extract contains all solutions except the extract, and blanks contain all solutions except β-carotene. Antioxidant activity measured by this test was determined by the following formula:

β-carotene Bleaching Inhibition % = [1 - (E0 - E120) / (C0 - C120)] × 100

Where:

E0: absorbance of the solution containing the extract before incubation;

E120: absorbance of the solution containing the extract after incubation for 120 min at 50°C;

C0: absorbance of the control (without the extract) before incubation;

C120: absorbance of the control (without the extract) after incubation for 120 min at 50°C.

III.3. Ferric Reducing Power

The reducing power of the extracts was determined according to the method of Yildirim et al. (2001). Specifically, 0.5 ml of an extract at desired concentrations was mixed with 1.25 ml of 0.2 M phosphate buffer, pH = 6.6, and 1.25 ml of potassium ferricyanide [K3Fe(CN)6] at 10 g/1. The mixtures were incubated for 30 minutes at 50°C. After incubation, aliquots of 1.25 ml of 10% trichloroacetic acid were added to the mixtures before centrifugation for 10 min at 3000×g. A volume of 1.25 ml of supernatant solution was mixed with 1.25 ml of distilled water and 0.25 ml of iron chloride (FeCl3) at 10 g/1. The absorbance was measured at 700 nm after 10 min. Higher

absorbance indicates better iron reduction. The addition of ferricyanide to the extracts allows its reduction to ferrocynide if there is antioxidant activity. The detection of the latter is performed by the addition of FeCl3. Trichloroacetic acid was added to stop the reaction. High absorbance indicates a significant amount of ferrocynide.

Results and discussions

I. Physicochemical Composition

The composition of the cladodes varies depending on edaphic factors, cultivation site, seasons, and plant age.

1. Yield

The yield of dried prickly pear cladodes before powder transformation is 6.7%. This result surpasses that reported by Msaddak (2018) and by Ait Maamar and Ait Abdelouahab (2019), which are approximately 5.20% and 5.7% respectively. Opuntia ficus-indica cladodes are high in water content, hence their lower drying yield (Ayadi et al., 2009).

2. Moisture Content

The average moisture content of the studied plant's cladodes is 93.25% of fresh weight. Our results align with those reported by Ayadi et al. (2009), Boutakiout (2017), and Valente et al. (2010) (90.00% to 95.68%), and are higher compared to results from Maataoui et al. (2002), Touré et al. (2016), and Msaddak (2018). Generally, cladode moisture content ranges between 88% and 95% (Murillo-Amador et al., 2002).

The high water proportion in the cladodes is due to the cactus being a succulent xerophytic plant capable of storing large amounts of water. This makes Opuntia valuable for resolving livestock watering issues in arid and semi-arid regions (Lee et al., 2003).

3. Ash Content

Ash represents the mineral residue of the plant after incineration, indicating the overall mineral content. The total ash content obtained is 22.5% ± 0.5% of dry matter (DM), higher than that obtained by Malainine et al. (2001) and Boutakiout (2017), and lower than that described by Msaddak (2018), Touré et al. (2016), and Hernandez-Urbiola (2011).

For two-year-old cladodes, this content exceeds 25% of dry matter (Nefzaoui and Chermiti, 1991; Ayadi et al., 2009). Variability in these results can be attributed to geographical location, climate, and soil physical properties.

4. Mineral Element Content

The results of mineral elements measured in this sample are presented in Table 01:

Potassium

The average potassium content is approximately 1187mg/100g DM. This value is lower than those reported by Messadek (2018) and Ayadi et al. (2009) (2266.50mg/100g and 3300mg/100g

respectively), possibly due to climatic conditions, soil, and genetic factors. These mineral quantities serve as a good mineral supplement for juices or other commercial foods. Potassium was also the most abundant mineral element in Mexican Opuntia cladodes (Ayadi et al., 2009).

Sodium

The sodium content in Opuntia ficus-indica cladodes is around 459mg/100g, higher than that found by Messadek (2018) (146.92mg/100g) and Ayadi et al. (2009) (200mg/100g). Tegegne (2001) reported a content of approximately 60mg/100g. However, this mineral composition varies according to species, regions, and physiological stage (Stintzing and Reinhold, 2005; Ayadi, 2009; Nefzaoui and Ben Salem, 1995).

Calcium

The calcium content is 711mg/100g according to Table 01. Comparing our results with those previously published by other authors, they fall within the range found by Kader (2002) and Piga (2004) (564-756mg/100g) and are higher than those reported by Messadek (2018) (446.20mg/100g) and Stintzing and Reinhold (2005) (310-630mg/100g).

Table 01: Mineral Element Content of Opuntia ficus-indica Cladodes

Mineral	Content in mg/100g
Potassium (K)	711
Sodium (Na)	1187
Calcium (ca)	459

I.5. Lipids

As in most forages, the fat content is low. This content, which averages 1.3 to 2.3 g/100 g of dry matter, decreases with age.The fat content of the studied plant, with a rate of 0.3%, appears very low compared to that reported in the literature by Msaddak (2018). However, the spiny and spineless cladodes of 2-year-olds studied by Ayadi (2009) show higher fat contents of 3.95% and 4.69%, respectively. This difference in content is even more significant in the spineless and spiny cladodes studied by Rodriguez-Felix et al. (1988) and Malainine et al. (2003), with rates of 2.4% and 7.2% per 100 g of dry matter, respectively.

In general, forages have a low fat content, and Opuntia is no exception. Its content, which is 1.96% for one-year-old cladodes, decreases with age (Kartez, 1996). This content also varies according to periods; according to Retamal et al. (1987), the highest contents are observed at the beginning of fruiting.

Table 02: Physicochemical composition of prickly pear cladode powder

Parameters	Prickly Pear Cladodes
Drying yield	6,7%
Moisture	93,25% (FW)
Ash	22,5g/100g (DM)
Lipid	0,3g/100g (DM)
Carbohydrates (glucose)	233,33 mg/100g (DM)
Chlorophyll	447,5 µg/100g (DM)

I.6. Carbohydrates

Carbohydrates, also known as sugars, are stored as starch in plants. They act as natural laxatives, aiding digestion, and constitute the main source of energy utilized by the body.

According to Table 03, the total carbohydrate content averages approximately 233.33 mg/100g. The carbohydrate content in the cladodes was lower compared to that found by Hernandez-Urbiola from Mexico (60.77%) (Hernandez-Urbiola, 2011) and that reported by Msaddak in 2018 (59.16%).

Research conducted in Tunisia (Ayadi, 2009) confirms that fructose is the most important soluble sugar. Reducing sugars represent 0.64 to over 0.88 g/100 g of fresh cladodes, increasing during growth and according to varieties (Rodriguez-Felix and Cantwell, 1988). Meanwhile, Monjauze reported this rate to be 1.90% and 5.10% of DM for cladodes aged less than one year and two years under North African conditions (Hadj Sadok, 2010).Opuntia ficus-indica cladodes are rich in sugar, explaining their use as livestock feed.

I.7. Chlorophyll

Table 02 shows that the total chlorophyll content, a natural pigment, is lower at 447.5 µg/100g of dry matter compared to that reported by Msaddak in 2018. Differences could be associated with the season of collection and geographical variations.

Total chlorophyll in the cladodes is estimated at 12.5 mg/100g of fresh weight, with chlorophyll (a) content at 9.5 mg surpassing chlorophyll (b) at 3.0 mg (Guevara et al., 2001). Ayadi et al. (2009) found total chlorophyll contents ranging from 13.59 mg/100 g of fresh weight to 12.22 mg/100 g of fresh weight for spineless and spiny cladodes.

II. Phytochemical Analyses and Antioxidant Properties

Phenolic compounds such as flavonoids and phenolic acids are primarily responsible for antioxidant properties. Phenolic compounds exhibit significant antioxidant activity through hydrogen or electron-donating reactivity (Dziki et al., 2014).

II.1. Yield

The extraction yield and extract color are represented in Table 03 below:

Table 03: Yield and color of different Opuntia ficus-indica extracts

Extract	Yield	Color
Methanolic	31.4%	Dark green
Ethanol	3%	Dark green
Hexane	13.86%	Light green
Aqueous	11.84%	Brown

Is important to emphasize that the method used (choice of solvents), as well as the conditions under which the extraction is performed, all affect the total content of phenols and flavonoids.

According to the results reported in Table 14, it is evident that the methanolic extract is the most efficient extraction solvent (31.4%), while the ethanolic extract yielded a lower yield (3%). The variation in extract concentration is due to the difference in polarity of the solvent used for extraction.

II.2. Total Polyphenols

For extracts of the studied plant, Opuntia F.I., we observed variability in total phenolic contents. The results illustrated in Figure 13 indicate that the ethanolic extract yielded the highest polyphenol content (48.515 mg GAE/g), followed by the methanolic extract with a polyphenol content of approximately 44.17 mg GAE/g, then the aqueous extracts and hexane extract with contents equal to 33.36 mg GAE/g and 26.39 mg GAE/g respectively. The results we obtained are higher than those cited in the literature (Messadek, 2018) (2485 mg GAE/100 g).

According to Ayadi (2009), in two-year-old cladodes, the polyphenol content is higher, reaching 91.04 mg/100g of fresh weight in spiny cladodes and 73.9 mg of fresh weight in spineless cladodes. Hadj Sadok in 2010 reported that this varies between 23.4 mg/100g to 41.6 mg/100g of fresh weight.

We observe a multitude of variations in the results of different studies. This phenomenon may be due to several factors (precipitation, light, soil type, topography, genetics, and maturity level) (Bouzoubaâ et al., 2014).

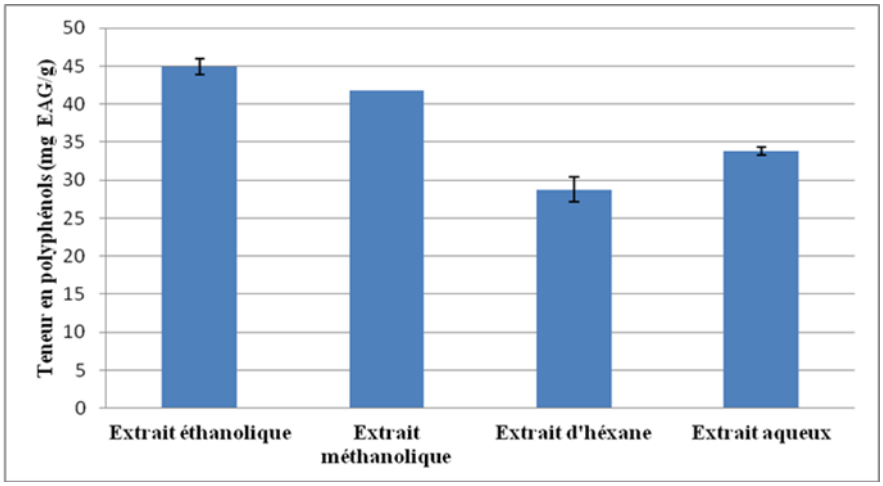

Figure 02: Total polyphenol content of different extracts from O.F.I. cladodes.

Several studies have been conducted on the extraction of Barbary fig cladodes, for example, using acetonitrile, acetone, ethyl acetate, dichloromethane, hexane, methanol, and water. These studies have shown that most phenolic compounds are found in the ethanolic extract.

II.3. Determination of flavonoid content

The results of the flavonoid content of four extracts are depicted in the figure below:

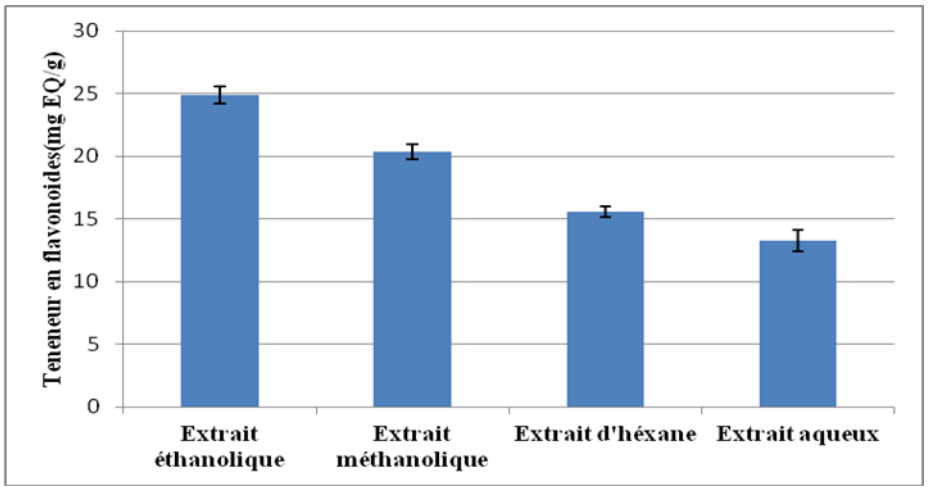

Figure03: Flavonoid content of different extracts from O.F.I. cladodes.

According to Figure 03, it can be observed that the flavonoid content ranges from 13.25 to 24.84 mg EQ/g; the highest value was recorded in the ethanolic extract, while the lowest content was observed in the aqueous extract. These values obtained were higher than those reported in the literature (Messadek, 2018) (1063 mg EQ/100 g). In the study conducted by Touré et al. (2015), the cladode contained a flavonoid proportion of approximately 73.53 mg EQ/100g.

Several parameters can also affect the stability of flavonoids. Light, pH, temperature, solvent nature, enzyme presence, oxidants, and metallic ions promote flavonoid degradation (Medjadji, 2012).

III. Antioxidant Activity

Several methods can be used to estimate antioxidant activity. Some of them rely on the reducing capacity of a compound as a significant indicator of its antioxidant potential, while others rely on the measurement of a substance's ability to trap radical compounds (Javanmardi et al., 2003; Marc et al., 2004).

In this study, the antioxidant activity of extracts from the studied plant's cladodes was determined using three different methods:

- The first method is the evaluation of the antiradical power by measuring the percentage of DPPH radical neutralization by the antioxidants present in the studied cladode extracts.

- The second method involves the capacity of different extracts to inhibit β-carotene bleaching and neutralize linoleic acid.

- The third method involves estimating the reducing power, which measures the extracts' ability to reduce metallic ions (ferric to ferrous iron).

III.1. Determination of anti-radical activity against DPPH radical

Figure 04 presents the anti-radical activity of ethanolic, methanolic, hexane, and aqueous extracts of Barbary fig cladodes as well as the reference molecule ascorbic acid. According to the figure, it can be noted that the anti-radical activities of different extracts are quite significant; all analyzed samples eliminated all free radicals. The methanolic and ethanolic extracts are noted for their significant antioxidant power, with respective IC50 values of 4.816 and 4.920 µg/ml, relatively similar to that of ascorbic acid, whose value is around 4.161 µg/ml.

These results indicate that the methanolic extract is the most active and capable of acting at a low dose.

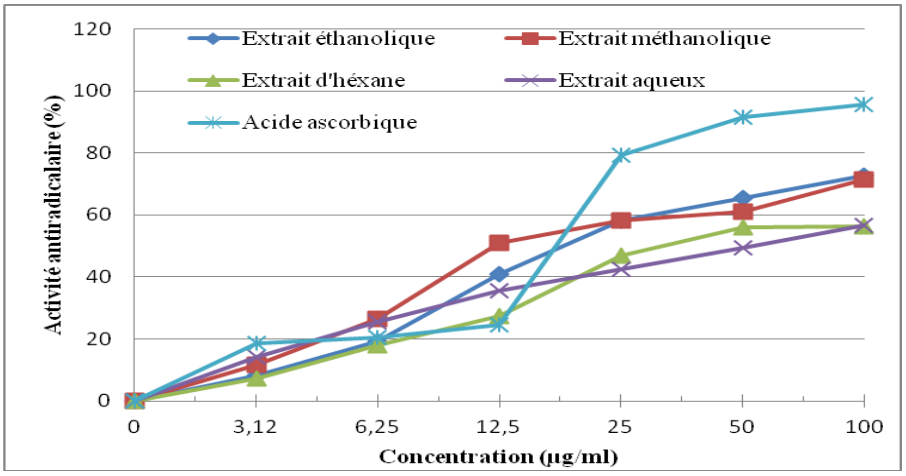

Figure 04: Antioxidant activities of various extracts from Barbary fig cladode powder.

Compared to other studies, our results align with those obtained by Bari et al. (2012) on Opuntia Monacantha cladode extracts, where the percentages of DPPH radical inhibition are higher in fractions rich in polyphenols and flavonoids. The reported IC50 inhibitory concentration value by Messadek (2018) is around 1.45 mg/ml. Previous works have mentioned a positive linear correlation between anti-radical activity and total phenol content (Shahidi et al., 2006). In fact, as the concentration of phenolic compounds increases, the anti-radical activity also increases, which is consistent with our results. These studies suggest that phenolic compounds are capable of neutralizing free radicals by donating a hydrogen atom to them.

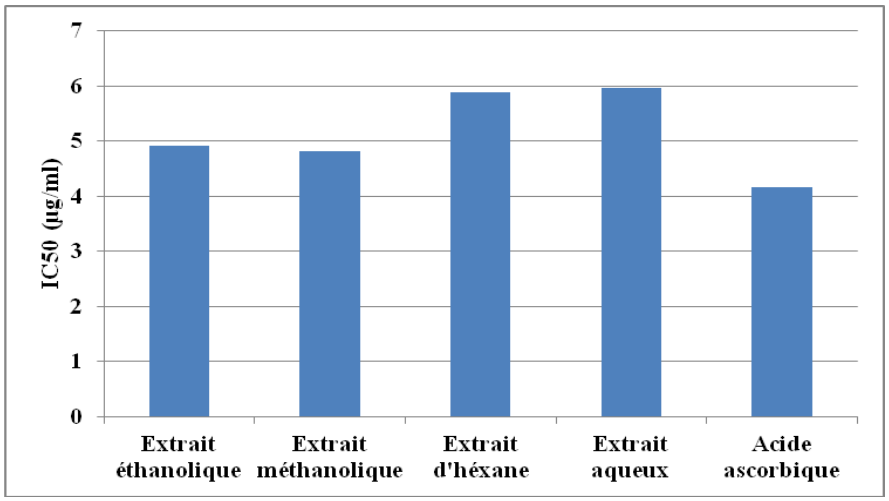

Figure 05: IC50 inhibitory concentrations of the DPPH test.

In the histogram (Figure 05), we can rank the extracts in decreasing order of reactivity: Ascorbic acid > methanolic extract > ethanolic extract > hexane extract > aqueous extract. The activities of our extracts are good, except for the hexane and aqueous extracts which exhibit low activity. This suggests that this portion of our extracts is poor in phenolic compounds, which are responsible for antioxidant activity.

III.2. β-carotene/linoleic acid bleaching assay

In this method, antioxidant activity is measured by a compound's ability to reduce the coupled oxidation of linoleic acid and β-carotene in an emulsified aqueous system (Abdille et al., 2005).

The capacity of extracts from the studied plant's cladodes to neutralize linoleic acid peroxidation products is reported in Figure 06.

The results show that the addition of various extracts of Barbary fig to the linoleic acid/β-carotene mixture prevents the bleaching of the latter, indicating the richness of the extracts in free radical scavenging antioxidants. Therefore, the various extracts are capable of inhibiting β-carotene bleaching in a dose-dependent manner.

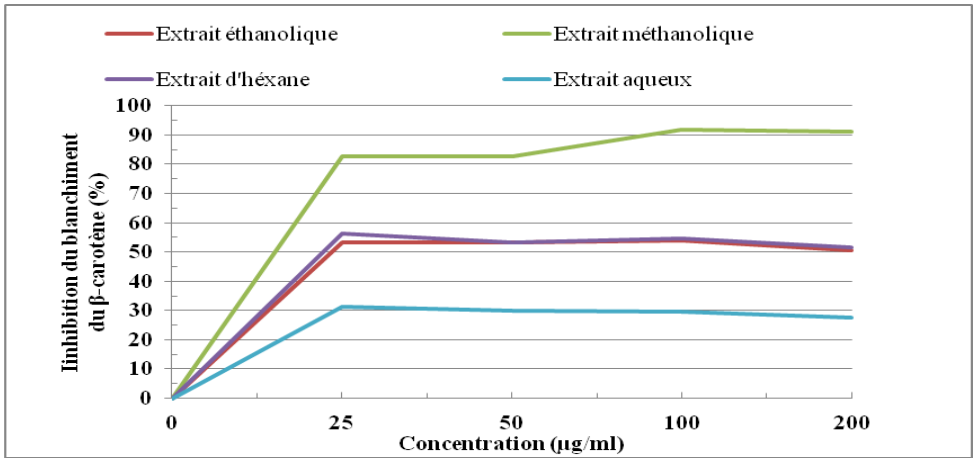

Figure 06: β-carotene/linoleic acid bleaching assay of O.F.I. extracts.

Indeed, the IC50 values, defined as the concentrations of extracts required to achieve 50% inhibition of β-carotene peroxidation, are 1.970 - 3.680 - 3.7651 and 7.916 µg/ml for methanolic, ethanolic, hexane, and aqueous extracts respectively (Figure 23).

Messadek (2018) reported a higher IC50 value (0.84 mg/ml) compared to the results of this study.

These results highlight the effectiveness of antioxidants present in these extracts in trapping free radicals in an emulsified environment. The results obtained are explained by the richness of the extracts in phenolic compounds, confirmed by Anwar et al. (2007), who stated that the presence of phenolic antioxidants prevents or reduces the bleaching of β-carotene by trapping peroxide radicals.

III.3. Ferric Reducing Power Assay

Ferric reducing power is often used as an indicator of a compound's ability to donate an electron. Many authors coincide the reducing capacity of a compound as a significant indicator of its antioxidant potential, which is an important mechanism that expresses the antioxidant action of phenolic compounds (Wang et al., 2008).In our study, we tested various extracts from Barbary fig cladodes using the FRAP method, and the results obtained are illustrated in Figure 07.

Analysis of the results reveals that the reducing power differs from one extract to another and that the ferrous ion reduction capacity is proportional to the increase in sample concentration. Therefore, the reducing power depends on the extract concentration.

The results presented in Figure 24 show that all our extracts exhibit antioxidant activities. Thus, we can deduce that all extracts from Opuntia ficus indica cladodes have the ability to reduce iron. The evaluation of the reducing power of the analyzed extracts also showed a better activity of the methanolic extract of the cladodes compared to other extracts, as shown in Figure 07. This variation is due to the variability in the content of active compounds in the extracts.

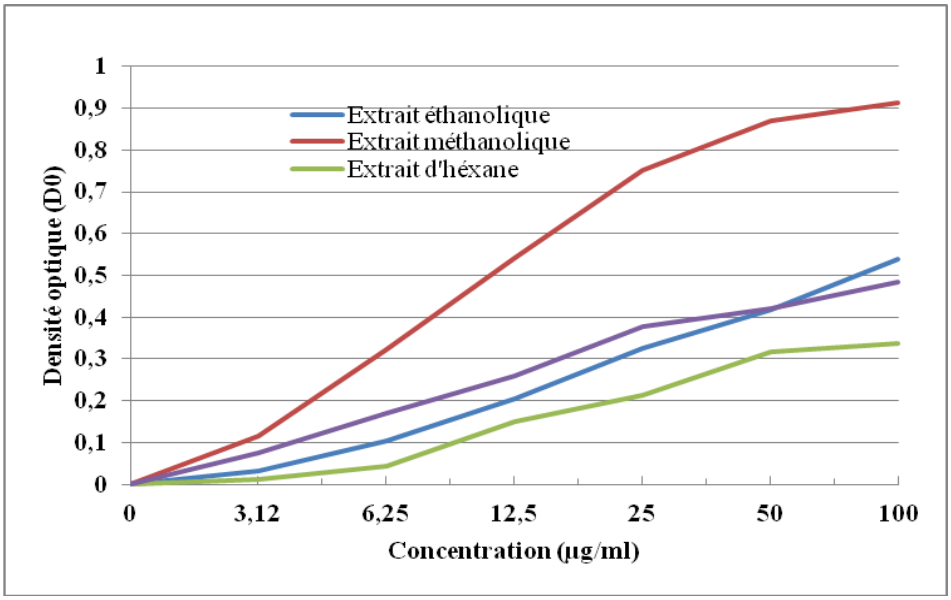

Figure 07: Ferric Reducing Power of Different Extracts from O.F.I. Cladodes

The CE50 values, defined as the concentrations of extracts providing 0.5 absorbance value at 700 nm, are 3.98 - 6.87 - 6.91 and 9.41 µg/ml for methanolic, ethanolic, aqueous, and hexane extracts, respectively (Figure 24).

For comparative purposes, the reducing power of the ethanolic extract of O.F.I. cladodes (CE50 = 0.40 mg/ml) is significantly lower than that found in our samples (Messadek, 2018).

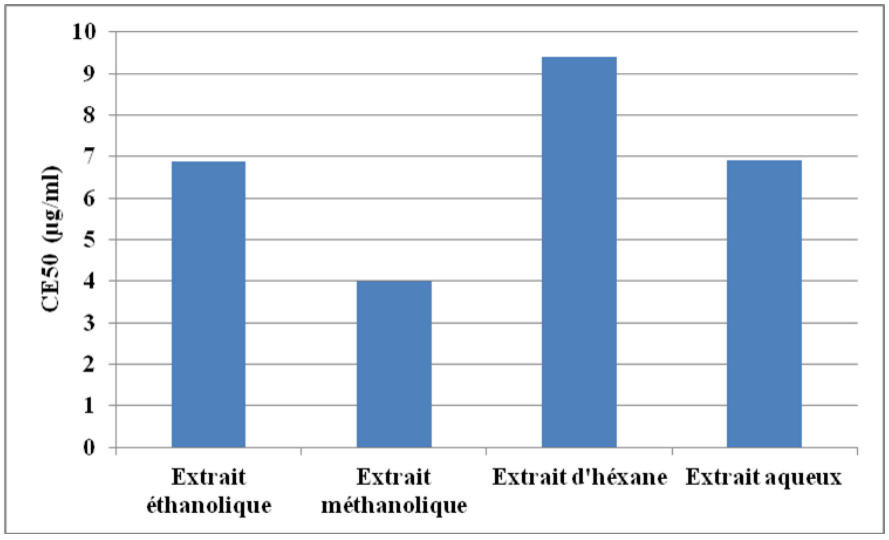

Figure 08: IC50 Values of Different Extracts from O.F.I. Cladodes

In this study, we aimed to measure the antioxidant activities of various extracts from O.F.I. cladodes using complementary methods such as anti-radical activity (DPPH• assay), reducing power assay, and β-carotene bleaching assay coupled with linoleic acid auto-oxidation. Generally, the use of

multiple tests provides us with a better understanding of the antioxidant activity of the studied extracts.

The results obtained showed that the methanolic extract of O.F.I. cladodes is more active than the other extracts in all three tests performed, with lower IC50 values (Figure 26). Moreover, at the same tested concentrations, the inhibition percentages obtained by the DPPH and FRAP assays are lower than those of the β-carotene bleaching method. This could be explained by the presence of substances that exhibit absorption bands at the same wavelength as the DPPH• radical, leading to an increase in absorbance (Sarr et al., 2015).

Therefore, the β-carotene method drew attention to the existence of three stages: "resting time, generation time, and exhaustion time", in which the methanolic extract was characterized by very high antioxidant activity (91.01%).

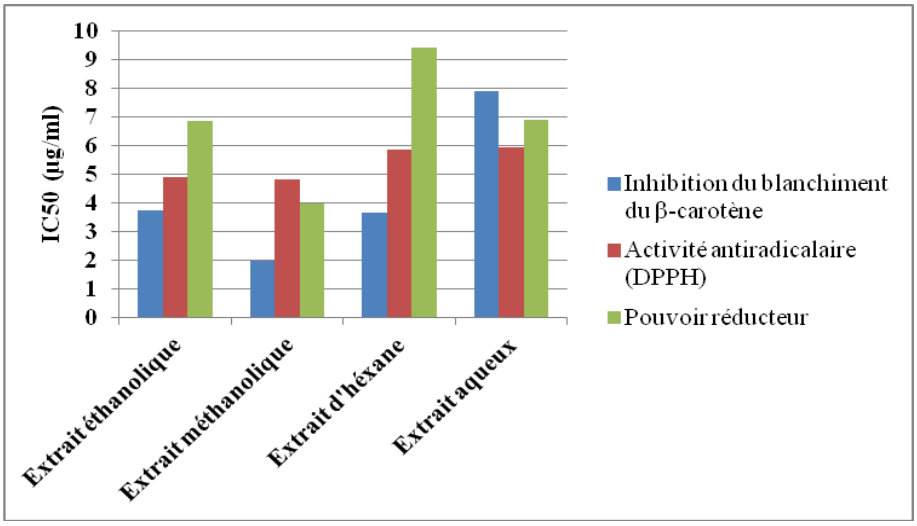

Figure 09: IC50 Values of Extracts According to Methods

Conclusion

The Barbary fig, a plant found on all continents, has shown diversity in behavior concerning environmental conditions (resistance to drought, frost, etc.) and in composition depending on the growth stage of the cladodes and the ripeness of the fruits. Barbary fig cladodes are rich in minerals, carbohydrates, ascorbic acid, polyphenols, and fibers. They contain bioactive components that have beneficial effects on health, fighting against several common diseases such as hyperglycemia and cholesterol reduction.In the general context of the food and therapeutic valorization of the Barbary fig, we focused on the cladodes of Opuntia ficus indica cultivated in the Northwestern Algerian region of Mascara for this study. This study aims to determine the chemical and phytochemical composition and evaluate the antioxidant activity of Opuntia ficus indica extracts.The analysis of the physicochemical parameters of the studied Barbary fig cladodes shows that they are generally very rich in water, with an average moisture content of 93.25%, and have a relatively low yield of 6.7%. The total ash content obtained contains a 22.5% rate of dry matter (DM), with sodium being the most abundant mineral in the Barbary fig cladodes, followed by potassium and calcium. The plant's fat content, at 0.3%, appears to be very low, while the Barbary fig cladodes are rich in sugar, and the total chlorophyll content is relatively low.The extraction yield of the plant's compounds is

conditioned by the choice of solvent and contact time. Therefore, it is important to note that methanol is the best extraction solvent with a higher yield compared to other solvents. Antioxidant assays revealed the richness of the cladodes in total polyphenols. Consequently, the methanolic extract shows very interesting antioxidant activity. This particular composition would confer beneficial properties, resulting in antioxidant effects. Despite various uses, Opuntia ficus-indica remains underexploited. It contains potentials that are still unknown, and uncovering them could give this tree a new boost in its context. Through this work, we hope to have made our modest contribution to the valorization of Barbary fig cladodes and to provide the population with a natural, effective, and accessible product.

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