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Phytochemical screening and antioxidant activity of aqueous-ethanolic extracts of *Opuntia ficus indica*

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ABSTRACT

The aim of this work was to screen both the presence of bioactive secondary metabolites using petroleum ether and methanolic extracts of green fruit (seed and skin) and cladode of Opuntia ficus indica, the quantitative determination of total phenolics, flavonoids contents, and various in vitro antioxidant activities (ferric reducing antioxidant power and 2,2-diphenyl-1-picrylhydrazyl) of hydro-ethanolic extracts. The tests of phytochemical characterization of dry matter plant including sterols/terpenes, polyphenols, flavonoids, tannins, anthraquinones, alkaloids and saponins were used in the present study. The active antioxidant, phenolic contents and flavonoids contents were determined using colorimetric method. The phytochemical characterization of Opuntia ficus indica dry seed, skin and cladode various extracts showed the presence of phenolic compounds, alkaloids (seed extract only) and saponins (skin extract only), against anthraquinones were not detected. The total phenolic contents, expressed as mg of gallic acid equivalent (GAE) per 100 g of dry matter, was found to be (73.12±1.26) mg GAE/100 g, (935.21±103.02) GAE/100 g and (390.90±1 4.50) GAE/100 g for seed, skin and cladode respectively. The radical scavenging activity, expressed in terms IC_{50} was found to be (185.85±3.57) µg/ml, (365.87±11.95) µg/ml and (1208.75±179.21) µg/ml respectively. Result for all aqueous ethanolic extracts using FRAP method was (50.06±1.07) mg AAE/100g, (318.15±1.62) mg AAE/100 g and (120.90±4.80) mg AAE/100g for seed, skin and cladode respectively. This work suggests the possibility of using dry seed, skin and cladode extracts of Opuntia ficus indica for the prevention of oxidative stress, for it has potential antioxidant activity.

Key words: Phenolic compounds, Flavonoids contents, seed, skin, cladode

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INTRODUCTION

The imbalance between oxidant/antioxidant in favor of oxidants is named “oxidative stress” [1]. Free radicals and reactive oxygen species (ROS) are highly reactive molecules and can damage cell structures including nucleic acids, carbohydrates, lipids, proteins [2]. Oxidative stress contributes to many pathologies such as: cardiovascular diseases, atherosclerosis, cancer, diabetes, neurodegenerative diseases.

The research done on antioxidants in vegetable products has received much attention [3, 4, 5, 6, 7], and efforts have been made to valorize new natural resources for active antioxidant compounds, especially phenolic compounds [8, 9]. The present antioxidant from plant matrix helps to prevent oxidative damage from occurring in the body.

The antioxidant methods including *in vitro* tests, DPPH and FRAP have been frequently used to predict antioxidant activity in plant extracts [10, 11].

The aim of the present study was to investigate qualitatively the presence of phytochemical contents of *Opuntia ficus indica* extracts (seed, skin and cladode) and then to evaluate their antioxidant activities. We undertake to provide an appropriate base for further exploitation of these fruit and cladode as sources of natural antioxidant for oxidative stress applications.

EXPERIMENTAL SECTION

2.1. Plant material

The plant samples (green fruits and cladodes) were collected in July 2014 in the region of Temara, South of Rabat (Morocco).

The fresh plant parts were washed with running water, air-dried. The prickly pear fruits was hand peeled. The skin and seeds were separated from the juicy pulp, washed abundantly with distilled water then dried at room temperature. Cladodes were washed by distilled water, dried under shade. The dried plant materials were grounded into fine powder using the electric blender.

The plant was identified in Laboratory of Pharmacognosy, Faculty of Medicine and Pharmacy, University Mohammed V, Morocco. A voucher specimen of the plant was deposited in the Laboratory.

2.2. Phytochemical characterization

Qualitative tests for the screening of certain phytochemical compounds were performed separately on the petroleum ether and methanol extracts of fruits and cladodes of *Opuntia ficus indica (OFI)* using standard procedures by the methods of Harvone [12], Trease and Evans [13] as reported by Buvaneswari [14].

2.3. Phenolic extraction

The solid-liquid extraction method reported by Nawaz et *al.* [15] was used to extract the phenolic compounds from defatted skin, cladode, seed powders. Thirty grams of powdered samples were extracted by 100 ml of aqueous ethanol (ethanol: water, 70:30 v/v). The solution was subject to agitation during 1hour at ambient temperature in darkness and then filtered. The extraction procedure was repeated twice in the same conditions. All filtrates were combined, evaporated at 40°C under vacuum using a Büchi 461 rotary evaporator.

The hydro-alcohol extraction value was determined as follows: % yield = $[(M_1-M_0)/M_2]x100$. Where M_0 is the weight of the empty flask (g), M_1 is the weight of the flask after evaporation (g) and M_2 is the weight of the seeds powder (g). The obtained extract was kept away from light at low temperature [16].

2.3.1. Determination of total phenolic contents

The Folin Ciocalteu method [17] was adopted with small modification to determine the total phenolic contents in different extracts. To the aliquot of 0.25 ml was mixed with 1.25 ml Folin-Ciocalteu reagent, diluted ten times. After that, 1 ml of sodium carbonate (7.5%) was added. The mixture was incubated in darkness for 30 min. The absorbance was measured at 765 nm against a blank. The total phenolic contents was expressed as gallic acid equivalents (GAE) in milligrams per 100 g dry material.

2.3.2. Determination of flavonoids contents

The flavonoids compounds content were determined as described by Chougui [16]. In summary, 1.5 ml of extract was added to 1.5 ml of $AlCl_3$ reagent (2%). After 30 min of incubation in darkness, the absorbance was readed at 430 nm against a blank. Quercetin was used as standard for the calibration curve. The results are expressed as mg equivalent of quercetin (QE) per 100 g of dry matter.

2.3.3. Determination of antioxidant activity

Reducing power assay

The reducing power was determined using method of Jayanthi et Lalitha [18]. Five hundred microlitres of sample extract were added to 2.50 ml of phosphate buffer (0.2 M, pH6.6), 2.50 ml ferricyanide potassium and 2.50 ml trichloro-acetic acid (10%). The mixture was incubated at 50°C for 20 min. After incubation, 2.50 ml of the mixture were picked and added to 2.50 ml of distilled water and 0.50 ml of ferric chloride (0.1%). The absorbance was readed at 700 nm. The results were expressed as mg ascorbic acid equivalent (AAE) per 100 g of dry matter.

DPPH scavenging assay

The method used in this assay was described by Brand-Williams [19] with a few modifications. The test was performed by mixing 750 µl of extract or standard with 1.75 ml of DPPH dissolved in methanol (0.02 g/ l). The mixture was incubated in darkness for 30 min, then the absorbance was measured at 515 nm against a control. Each

reaction was performed in triplicate. The free radical inhibition was calculated as a percentage of inhibition of DPPH, given by the formula:

% inhibition= $[(A_c-A_e)/A_c] \times 100$

Where A_c is the absorbance of the control and A_e the absorbance of the aqueous ethanolic extracts sample.

Radical scavenging activity was expressed as EC_{50}, the concentration that inhibited 50% of DPPH radical. EC_{50} was calculated from the graph of DPPH inhibition percentage against extract concentration.

Statistical analysis

Data are expressed as mean ± standard deviation from three separate observations. For in vitro antioxidant (FRAP and DPPH) and Total phenol compounds assays (phenol and flavonoid) one way ANOVA test was used to analyze the significative of the difference between various extracts studied ($P < 0.05$). IC_{50}, was graphically determined by a linear regression method.

RESULTS AND DISCUSSION

Phytochemical characterization

The qualitative examination of six extracts was summarized in the table 1. Different varieties of phytochemical were included such as Sterols/Terpenes, Flavonoids, Tannins, Anthraquinones, Alkaloids and Saponins. The results obtained from different organs of *OFI* are represented respectively.

Table 1: Screening of some phytochemicals in different parts of of *Opuntia ficus indica*

Constituents	Petroleum ether			Methanol		
	Seed	Skin	Cladode	Seed	Skin	Cladode
Sterols/ Terpenes	-	-	-	+	+	+
Flavonoids	-	-	-	+	+	+
Tannins	-	-	-	+	+	+
Anthraquinones	-	-	-	-	-	-
Alkaloids	-	-	-	+	-	-
Saponins	-	-	-	-	+	-

(+) indicate presence; (–) indicate absence

Total phenolic contents and flavonoids contents in hydro-ethanolic extracts four seed, skin and cladode

The total phenolic contents and flavonoids contents among the three extracts were shown in table 2 and expressed in term of gallic acid equivalent and quercetin equivalent using the standard curves equations $y = 10.98x + 0.0069$, $R^2 = 0.9999$ and $y = 38.921x + 0.0027$, $R^2 = 0.9998$. The total phenolic contents in seed, skin and cladode extracts of *Opuntia ficus indica* showed different results varied from 73.12±1.26 to 935.21±103.02 mg GAE/100 g and 35.42±0.38 to 83.42±0.46 mg QE/100 g.

Reducing power activities

The presence of extract causes reduction of the Fe^{3+}; Fe^{2+} is monitored by measuring the formation of Perl's Prussian blue at 700 nm [20]. The ferric reducing power may serve as a significant indicator of the antioxidant potential. The reducing capabilities of aqueous ethanol extracts of *OFI* are shown in figure 2. Absorbance at 700 nm showed greater reducing power. At different concentrations 0.05, 0.1, 0.5, 1.0 and 2.0 mg/ml; the reducing power of hydro-ethanolic extracts (skin, seed and cladode) of *OFI* were found to be 318.15±1.62, 50.06±1.06, 120.90±4.80 mg AAE/100 g, respectively.

DPPH scavenging activities

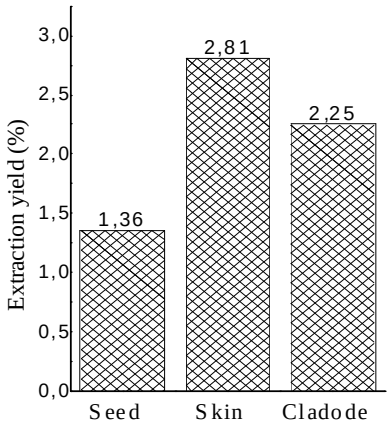
In the DPPH free radical scavenging activity, hydro-ethanolic extracts (fruits and cladode) were evaluated for their free radical scavenging activity with quercetin as standard. A different concentrations tested 1, 10, 20, 100 and 200 µg/ml. The antioxidant activity of *Opuntia ficus indica* extracts of different matrix are compared and shown in figure 3. The free radical scavenging effect of aqueous ethanolic extracts of *OFI* was determined using the DPPH method. The extracts of seed, skin and cladode showed IC_{50} values of 185.85±3.57 µg/ml, 365.87±11.95 µg/ml and 1208.75±179.21 µg/ml respectively; where the IC_{50} values of quercetin is 51.16±2.61 µg/ml as a results, there is higher difference in the antioxidant activity.

Table 2: Total phenolic and flavonoid contents of *Opuntia ficus indica*

Plant material	Total phenolic content (mg GAE/100 g)	Total flavonoid content (mg QE/ 100 g)
Seed	73.12±1.26	35.42±0.38
Skin	935.21±103.02	83.42±0.46
Cladode	390.90±14.50	73.53±6.30

GAE = Gallic acid equivalent; QE = Quercetin equivalent

Figure 1: The extraction yield of *Opuntia ficus indica*



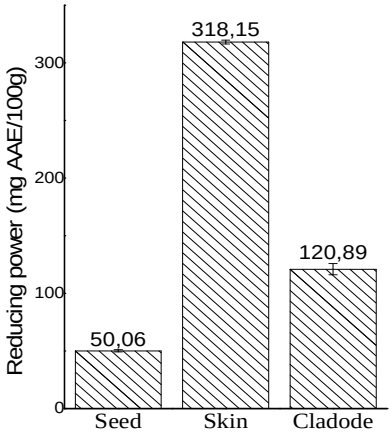


Figure 1: Results of power reducing activities of *Opuntia ficus indica*

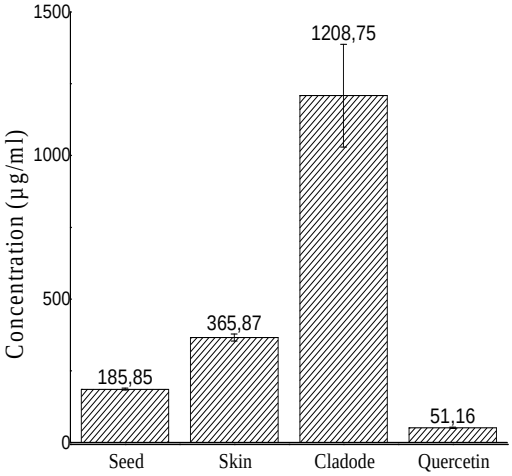


Figure 2: The DPPH radical scavenging capacity (IC_{50}) values of aqueous ethanolic extracts of *Opuntia ficus indica* and quercetin

At the 0.05 level, the population means for all parameters (total phenolic contents, flavonoids contents, reducing power and DPPH radical scavenging) are significantly different ($p<0.01$).

Yields of different extracts obtained by maceration method are presented in figure 1. The hydro-ethanolic extracts have been estimated for *OFI* seed, skin and cladode powders. The results showed polar components contents that varied from 1.36% to 2.81% (w/w) dry matter (DM). The skin from the fruit had the highest hydro-alchohol extracts contents, followed by the cladode one with 2.25%. The lowest percent was found in the seed. Variation in the yields of different extracts is attributed to the polarity of aqueous-ethanol mixture and solubility capacity of matrix components. This approach has been reported in literature [21, 22, 23, 24].

When compared to the petroleum ether extracts, the phytochemical screening of methanolic extracts of seed, skin and cladode of *OFI* revealed the presence of secondary metabolites. Anthraquinones were not found in all various extracts used for study. Alkaloids were found only in methanolic seed extract and Saponins only in methanolic skin extract. The results were partially similar to that reported by Hanane Dib et *al.* [25].

The phytochemical contents detected are known for their medicinal importance. For example, phenolic compounds derived from medicinal plants show biological activities like: antioxidant [26], antibacterial [27], antifungal [28], antiparasitic [29] and anti-inflammatory [30].

Phenolic compounds are secondary metabolic products widely distributed in plants; they have many biological and pharmacological properties that could provide protection against chronic disease. These compounds have more antioxidant effect. They are able to neutralize the effects of oxidative free radicals and reactive oxygen. The total polyphenols content (TPC) was estimated in different hydro-ethanolic extracts presented in table 1. The concentration of total phenolic was much more important in the skin compared to the cladode and seed extracts.

The amount found in the cladode was greater important to that recorded by Jaramillo-Flores et *al.* [31] (1589µg/g DW). However, it was higher to that noted by Bensadón et *al.* [32] (2.69 and 3.71 mg/g DW). Concerning the skin (peels), the level obtained in this study was higher to the literature of *OFI* recorded by Moussa-Ayoub et *al.* [33] (0.92 mg/100mg of DW). Paradoxally, the seed extract was less richer in phenolic component compared to the literature (2.76 and 1.54 mg/g) [32]. This difference is the result of the effect of a number of factors, the main ones are genetic, precipitation, light, topography, soil type and maturity [34].

The contents of total flavonoid, namely quercetin in hydro-ethanol extracts of *Opuntia ficus indica* fruits and cladode investigated are shown in table 1. The highest total flavonoid contents was found in skin, while seeds showed the lowest value. The flavonoid levels reported in this work were higher than values reported earlier in cactus pear fruits [35, 36]. Probably because we processed not only the skin but also the cladode and seed, which would be expected to show a higher phenolic contents.

Cactus pear fruits show a relatively high flavonoid contents, phytochemicals that contribute to antioxidant capacity and which have been extensively studied for their potential health benefits. These compounds are more antioxidants, since flavonoids and phenolic compounds in general are able to delay the prooxidative effects of proteins, DNA and lipids through the generation of stable radicals [37].

Reducing power of the extracts was found to be concentration dependent. The reducing power is based on the hydrogen donating ability. Reducing power of the compound may serve as a significant indicator of its potential antioxidant activity [38]. This activity increased linearly with concentration. Similar approach has been reported by Krishnaveni et *al.* in water fresh leaves extracts of *OFI* [39].

To evaluate the free radical scavenging activity, DPPH assay is widely used. At ambient temperature, DPPH is stable free radical which produces violet solution in methanol. At 517 nm DPPH shows strong absorption band in visible spectrum (deep violet colour).

From the results of DPPH, the aqueous ethanol seed extracts showed highest antioxidant activity compared to the hydro-ethanol skin and cladode extracts. In the DPPH system, the antioxidant activity of the skin of the *OFI* is similar with the results of Nizar Yeddes et *al.* [40], who used the DPPH antioxidant scavenging capacity to determine the antioxidant activity. With the increase in the concentration of extract the increase in the scavenging effect was observed. According to our observations, we opine that the strong activity of the extracts is due to the available hydroxyl group presented in the component [41].

CONCLUSION

This study evaluated the presence of tannins and flavonoids, determined the total phenolic compounds and flavonoids contents, and confirmed the antioxidant activities in aqueous ethanolic extracts of *OFI* using green fruit (seed and skin) and cladode. All this show that there is a potential antioxidant activity in this plant and reveals that the polar extracts of this species is promising sources for the search for new compounds that are useful in the prevention or treatment of diseases associated with oxidative stress.

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