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Email de l'auteur correspondant
mabroukilamia@yahoo.fr

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Evaluation of antioxidant capacity, phenol and flavonoid contents of *Opuntia streptacantha* and *Opuntia ficus indica* fruits pulp

Lamia Mabrouki,[a] Bouthayna Zougari,[b] Monia Bendhifi,[c] Mohamed A. Borgi, [c]

[a]*Laboratory of plant biotechnology, Faculty of Sciences of Sfax, 3000 Sfax. Tunisia*
[b] *Centre for agricultural research, 2200 Tozeur. Tunisia*
[c] *Research Unit of Macromolecular Biochemistry and Genetics, Faculty of Sciences of Gafsa, 2112 Gafsa. Tunisia*

Abstract

The aim of this study was to determine phenolic and flavonoid content and to evaluate antioxidant activity of fruit's pulp from 2 *Opuntia* species [*Opuntia ficus-indica* (OFI) and *Opuntia streptacantha* (OS)]. Samples were extracted with methanol and kept at 4°C until investigation. Total phenolics were determined by Folin-Ciocalteu method. Flavonoids were inspected based on the formation of flavonoid-aluminium complex. Antioxidant activity was evaluated using three different methods: the 2,2'-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method, the reducing power and the β-carotene bleaching test (BCB). *O. streptacantha* fruit extract contain more phenolics and flavonoids than *O. ficus-indica*. Using the DPPH method, the reducing power and the BCB, the highest activities were found for the extraction of OS fruit with the lowest EC_{50} values (5.76 ± 0.46, 2.01 ± 0.03 and 2.93 ± 0.77 mg/ml, respectively). The amount of phenolic and flavonoid compounds was implied as possible factor witch influenced the antioxidant power of the OFI and OS fruit extract.

Keywords: *Opuntia ficus-indica*; *Opuntia streptacantha*; antioxidant activity; total phenolic content; total flavonoid content

1. Introduction

Oxidative stress is defined as the disequilibrium between the increased levels of reactive oxygen species (ROS) and the low activity of antioxidant mechanisms. Oxidative stress has been recognized as playing a prominent role in curing many diseases processes like heart disease, inflammation, arthritis, immune system impairment, and cancer [1-3]. In human body, neutralization of these oxidizing molecules occurs through their interaction with a complex system of antioxidant processes mediated by endogenous antioxidative enzymes and substances. However, the native defense may be insufficient for serious or permanent oxidative stress. Hence, exogenous anti-oxidants interventions are necessary to balance the rate of the ROS and to stop their ominous effects in human body. Synthetic antioxidants such as butylatedhydroxyanisole (BHA) (butylatedhydroxytoluene (BHT) propylgallate (PG) and tert-butylhydroquinone (TBHQ) are very effective and are commonly used in food formulations [4]. Although, currently, pathological effects and carcinogenic potential of synthetic antioxidants have been carped [5]. Thus, in recent years, there has been increasing interest in finding natural alternatives especially of plant origin [6]. In this context, plants containing high concentration of numerous redox-active secondary metabolites or antioxidants, such as ascorbic acid, carotenoids, glutathione, tocopherols, polyphenols and others non-nutrient substances, received a great attention due to their ability to contract the deleterious effect of oxidative molecules [7,8]. In this line, in our laboratory, we have been interested to the study of antioxidant effect of plant extracts specially those which are widely distributed in Tunisia. Particular attention is given to the cactus plants. *Opuntia spp*., is the largest genus of the Cactaceae family, it involves more than 1500 known species [9]. The plant originating from Mexico is now spread in all the American hemispheres, South African countries and all over the

Mediterranean basin [10,11]. The presence of Crassulacean Acid Metabolism (CAM) in *Opuntia* which results in high water to dry matter conversion efficiency, make cactus pear (*Opuntia spp*.) perfect candidate for the production of both total biomass and fruits [12,13]. The therapeutic properties of the green parts of the plant, the cladodes, have very long been known in the traditional medicine [14, 15], however potential activities of the fruit, beyond nutritional benefits, have just been explored recently. But, certain cactus fruit belonging to *Opuntia* specie like *Opuntia ficus indica* fruits, have attracted the greatest attention of researches due to their commercial value whereas, others were less documented. Few researches have been reported on the antioxidant activity of *Opuntia streptacantha* fruit extracts. In view of these findings, the purpose of this work was to determine the phenolic and flavonoid content of *Opuntia ficus indica* and *Opuntia sterptacantha* fruit extract and to comparatively evaluate their *in vitro* antioxidant activity in three different model systems namely; DPPH radical scavenging activity, reducing power essay and β-carotene bleaching test.

2. Materials and methods

2.1. Chemicals

Butylated hydroxytoluene (BHT), ascorbic acid, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), 2,2 diphenyl-1-picrylhydrazyl (DPPH), Tween 40, β-Carotene, and linoleic acid were supplied from Sigma (Sigma, Aldrich). Gallic acid, rutin, Folin-Ciocalteu reagent, and trichloroacetic acid (TCA) were from Merck (Darmstadt, Germany). All other chemicals and reagents used were of analytical grade.

2.2. Plant material and preparation extract

The plant material used was fresh fruits of 2 *Opuntia* species: *Opuntia ficus-indica* (OFI) and *Opuntia streptacantha* (OS). Fruits were harvested at full maturity from Gafsa, Area in Tunisia. For the extraction procedure, *Opuntia* fruits were washed peeled then pulps were manually squeezed. For each fruit sample, 5g of squeezed pulps were added to 50 ml of 50% methanol and extracted for 24 h at room temperature, with magnetically stirring. The resultant suspension was centrifuged at 4500 × g for 10 min and the supernatant was filtered through a cotton plug followed by 0.45 µm microbial filter. The *O. ficus-indica* extract (OFIE) and *O. streptacantha* extract (OSE) were then stowed in a dark bottle at 4 °C for investigation. The plant authentication was made by the work of Bendhifi Zarroug *et al*., [16]

2.3. Determination of total phenolic contents (TPC)

Total phenolic content (TPC) was determined according to the Folin-Ciocalteau method with minor modifications [16]. Briefly, 100 µl of methanolic extracts was mixed with 900 µl of Folin-Ciocalteu reagent (diluted 1:10 with water). After 5 min, 0.75 ml of 7% sodium bicarbonate solution was added to the mixture and vortexed for 30 s. The above solution was then kept for incubation at room temperature for 90 min. Absorbance was measured at 765 nm in Analytik jena 40, spectrophotometer. The TPC was expressed as gallic acid equivalents (GAE) in comparison to a gallic acid calibration curve. All values were expressed as mean (mg of gallic acid equivalents/100 g of fresh weight) ± SD for 3 replications.

2.4. Determination of total flavonoid content

Favonoid in the methanolic pulp extracts was spectrophotometrically based on the formation of complex flavonoid-aluminium [17]. 1 ml of sample was mixed with 1 ml of 2% (w/v) aluminium chloride solution. After incubation at room temperature for 15 min, the absorbance of the reaction mixtures was measured at 430 nm against the blank. Rutin was used as the standard for preparing the calibration curve. The significant result was expressed as rutin equivalents (mg RE/100 g dry fresh weight) and all determinations were performed in triplicate.

2.5. Determination of DPPH (2,2'-diphenyl-1-picrylhydrazyl) radical-scavenging activity

The scavenging activity was monitored according to the method of Blios [18]. Various concentrations of *O. ficus-indica* and *O. streptacantha* methanolic extracts (1ml) were mixed with 1 ml of methanolic solution containing DPPH radicals (6.10^{-5} mol/l). The mixture was shaken vigorously and left to stand for 30 min at room temperature in the dark. Absorbance was read using a spectrophotometer at 517 nm. Trolox was used as the reference compound. The radical-scavenging activity (RSA) was calculated as a percentage of DPPH discoloration using the equation:

$$\%RSA = [(A0 - A1)/A0] \times 100$$

Where A_0 is the absorbance of the control (blank, without extract), and A_1 is the absorbance in the presence of the extract. The extract concentration providing 50% of radical scavenging activity (EC_{50}) was calculated from the graph of RSA percentage against extract concentration.

2.6. Determination of Ferric reducing power (FRAP)

The ferric reducing power (FRAP) of examined extracts was determined according to the method of Chu *et al.* [19].

1 ml of examined extracts in different concentrations (ranging from 1 to 5 mg/ml of stock solution) was mixed with 2.5 ml of potassium phosphate buffer (0.1 M, pH 6.6) and 2.5 ml of 1% (w/v) potassium ferricyanide.

The contents were incubated at 50 °C for 20 min then 2.5 ml of 10% (w/v) trichloroacetic acid were added. A 2.5 ml of the reaction mixture was combined with 2.5 ml of distilled water and 0.5 ml of a ferric chloride solution (0.1%, w/v) and incubated at 28 °C for 30 min. The absorbance was measured at 700 nm and compared to ascorbic acid (AA) which was used as positive control. Amplified absorbance of the reaction mixture designated increased reducing power. The extract concentration providing 0.5 of absorbance (EC50) was calculated from the graph of absorbance at 700 nm against extract concentration.

2.7. Determination of antioxidant activity by β-carotene bleaching method (BCB)

The antioxidant activity of OFI and OS fruit extract was evaluated using the β-carotene-linoleic acid assay [20]. Shortly, 2 mg of β-carotene was dissolved in 10 ml of chloroform and 1 ml of β-carotene solution was mixed with 40 mg of linoleic acid and 400 mg of Tween 40. Chloroform was completely evaporated using a vacuum evaporator. Then, 100 ml of oxygenated distilled water was added slowly to the mixture with a vigorous shaking, to form an emulsion. 2.5 ml of this reaction mixture was distributed to test-tubes and 250 µl of various concentrations (1, 1.5, 2, 2.5, 3…… 5 mg/ml) of extracts in methanol were added. The samples were put in a water bath at 50 °C for 120 min. The absorbance was measured at 470 nm at time zero and after 120 min of incubation with BHT, as reference antioxidant. The control sample contained 250 µl of solvent in instead of extract. Antioxidant activity was expressed as the percentage of inhibition of oxidation relative to the control and calculated as follows [21]:

$$\% \text{ Inhibition} = (AA(120) - AC(120) / AC(0) - AC(120)) \times 100$$

Where A A (120) is the absorbance of the sample at t = 120 min, A C (0) and A C (120) are the absorbance of control at t = 0 min and t = 120 min, respectively. Assays were performed in triplicate and the graph was plotted with the mean values. EC50 value, which is defined as extract concentration providing 50% inhibition, was obtained from the plot of inhibition percentage versus extract solutions concentrations.

3. Results

3.1. Total phenolic content

The total phenolic compound contents of fruit extracts were determined by the Folin Ciocalteu method and expressed as gallic acid equivalents (mg GAE/100g fresh weight basis). A significant difference was observed between the content of phenolic compounds of fruits belonging to both species. The methanolic pulp extract of *O. ficus-indica* exhibited about 54.33 ± 2.51 mg GAE/100 g extract whereas the total phenolic content was estimated to 104.66 ± 1.52 mg GAE/100g extract for *O. streptacantha*. The amount of total phenolic componnds in the OFI extract appears slightly higher than the value reported by Diaz *et al.*, [22], (45.2 ± 7.4 mg GAE/100g). For OS, our data are consistent with those obtained by Diaz *et al*,. [22] and José *et al.*, [23], who found comparable TPC contents in the *O. dillenii* and *O. xoconostle* fruit extracts (102 ± 0.03 and 117 ± 10 mg GAE/100g fresh weight, respectively). It is well known that the phenolic content contributed to the antioxidant activity of fruit extracts [7] and significant correlation between total phenols and antioxidant power have been obtained in many studies [24-26]. The phenolic compounds exhibit considerable free radical scavenging activities, through their reactivity as hydrogen- or electron-donating agents, and metal ion chelating properties [27]. These results suggest that the amounts of total phenolic compound contents may attribute for the *O. ficus-indica* and especially for the *O. streptacantha* extract a potential antioxidant activity.

3.2. Total flavonoid content

The total flavonoid content in the plant extracts was determined based on the formation of flavonoid-aluminium complex [17]. Our results showed that total flavonoid content detected in *O. streptacantha* extract (34.75 ± 1.89mg RE/100g extract) was higher than O. ficus-indica (22.47 ± 2.1mg RE/100g extract). Studies reported by Kutti [28] analyzed four *Opuntia* cactus pear fruit species (*O. ficus-indica*, *O. lindheimeri*, *O. streptacantha*, *and O. stricta*) and the author found that the predominant flavonoids in the fruits of *Opuntia* cactus pears consisted of quercetin, kaempferol and isorhamnetin, respectively. Based on various studies on *Opuntia* composition, significant difference in total flavonoid content was found in different parts of the fruit. The pericarp had the highest total flavonoid content value [29]. The flavonoids constitute about one-half of the 8000 so known polyphenols [30] and can occur as glucosides (including glucose side chains) or as aglycone (without glucose side chains). Flavonoids have been vital compounds for plants as they are involved in fruit and flowers coloration, photosensitization, energy transfer and the actions of plant growth hormones and growth regulators. In addition, these molecules provide to plants the protection from pathogens and UV radiation

[31]. In the human body, flavonoids are powerful antioxidants that help neutralize harmful free radicals and prevent oxidative stress and they are strongly correlated to antioxidant activity [32].

3.3. DPPH radical-scavenging activity

The DPPH is the most common synthetic radical to be used for the study of the contribution of structural characteristics to the radical scavenging activity of compounds [33, 34]. A freshly prepared DPPH solution exhibited a purple color showing a maximum absorption at 517 nm. The antioxidants, by donation of either hydrogen or electrons, reduce the DPPH radical to a yellow-colored compound, diphenylpicrylhydrazine, causing a decrease in absorbance at 517 nm. Positive DPPH test suggests that the samples were free radical scavengers. As shown in Table 1, The DPPH activity of OFI and OS extract were found to increase in a concentration-dependent manner. OS, by its lower EC50 value (5.76 ± 0.46 mg/ml), revealed the highest antiradical activities. The DPPH-scavenging activity of OSE (5.76 ± 0.46 mg/ml) and OFIE (8.63 ± 1.33 mg/ml) was respectively 2 and 3 fold lower than that of Trolox, a well-known antioxidant, (2.82 ± 0.02 mg/ml). Results obtained by José *et al.*, [23] demonstrated also the scavenging activity of OFI fruit extract on DPPH radicals. Moreover, earlier published data [35, 36] indicated positive DPPH test of OFI roots and stems extracts with EC50 values of 118.65 ± 2.51 µg/ml and 9.30 µg/ml, respectively.

Table 1
Scavenging effect (%) on DPPH radicals of *O. ficus-indica* and *O. streptacantha* methanolic extracts.[a]

	Sample concentration (mg ml^{-1})										
	1	2	3	4	5	6	7	8	9	10	EC_{50}
O. ficus-indica	11.54 ±1.51	18.46 ±1.89	20.89 ±3.46	22.15 ±1.97	24.62 ±0.75	33.85 ±0.72	38.46 ±2.06	44.63 ±3.01	52.31 ±2.69	53.85 ±1.01	8.63 ±1.33
O.streptacantha	24.6 ±2.03	29.23 ±1.12	35.69 ±1.51	40.00 ±0.43	43.68 ±0.74	50.77 ±1.27	55.38 ±1.98	60.15 ±0.52	71.54 ±1.11	82.00 ±0.88	5.76 ±0.46
Trolox	32.31 ±2.09	43.85 ±1.05	50.76 ±0.55	56.15 ±1.85	64.65 ±4.02	66.77 ±2.01	74.31 ±2.04	76.9 2±1.24	87.69 ±0.77	99.23 ±0.45	2.82 ±0.02

Trolox was used as reference standard.
[a] Values expressed are means ± S.D. of three parallel measurements.

3.4. Ferric reducing power (FRAP) assay

The ferric reducing power method is based on the transformation (reducing) of ferric–ferricyanide complex to the ferrous form depending on the presence of antioxidants [37]. The yellow color of the test solution changes to various shades of green and blue, depending on the reducing ability of each compound. A higher absorbance at 700 nm indicates a higher reducing power. Table 2 depicts the reducing power of OFIE, OSE and ascorbic acid (AA), used as standard. The reducing ability of the above mentioned samples increased with concentration. From the analysis of Table 2, we can conclude that the best reducing power being obtained with OSE reaching (0.86 ± 1.01) when tested at 5 mg/ml, even higher than the reducing power of the ascorbic acid standard (0.74 ± 0.55) for the same concentration. EC_{50} values were found to be 2.01 ± 0.03, 3.41 ± 0.11 and 4.71 ± 1.09 mg/ml for *O. streptacantha*, ascorbic acid and *O. ficus-indica*, respectively. The reducing properties, as perceptibly reported, are commonly related to the presence of reducers which employ their action by breaking the free radical chain through donating a hydrogen atom [38-40]. This would have the effect of converting free radicals to more stable products in the aim of prevention oxidative damage.

Table 2
Reducing power (absorbance of 700 nm) of *O.ficus-indica* and *O. streptacantha* methanolic extracts.[a]

	Sample concentration (mg ml $^{-1}$)									
	1	1.5	2	2.5	3	3.5	4	4.5	5	EC_{50}
O. ficus-indica	0.09±0.02	0.13±1.03	0.17±0.05	0.21±0.92	0.27±0.15	0. 33±2.00	0.38±0.88	0.47±0.55	0.53±2.02	4.71±1.09
O.streptacantha	0.32±0.92	0.44±1.12	0.50±0.06	0.59±0.13	0.63±0.08	0.68±1.15	0.74±0.05	0.81 ±0.02	0.86±1.01	2.01±0.03
Asscorbic acid	0.21±1.00	0.29±1.34	0.32±0.01	0.40±0.62	0.46±1.5	0.54±0.15	0.61±0.04	0.68 ±1.25	0.74±0.55	3.41±0.11

Ascorbic acid was used as reference standard.
[a] Values expressed are means ± S.D. of three parallel measurements.

3.5. β-Carotene bleaching (BCB) assay

	Sample concentration (mg ml^{-1})									
	1	1.5	2	2.5	3	3.5	4	4.5	5	EC_{50}
O. ficus- indica	11.88±2.71	16.54±1.82	21.25±1.12	24.00±1.34	27.53±0.56	38.62±2.72	48.12±0.96	51.37±1.11	57.37±1.02	4.00±1.04
O.streptacantha	18.75±5.00	24.37±1.13	32.3 ±2.94	35.42±3.56	49.35±3.61	55.87±1.19	63.12±0.92	65.62±1.88	71.00±2.21	2.93±0.77
BHT	22.52±0.91	32.87±0.49	41.25±0.63	46.87±1.56	55.75±1.39	62.76±0.73	68.82±0.05	73.76±2.02	84.25±1.01	2.71±0.05

Table 3
Antioxidant activity (%) of *O. ficus-indica* and *O. streptacantha* methanolic extracts by the β-carotene bleaching method.[a]
BHT was used as reference standard.
[a] Values expressed are means ± S.D. of three parallel measurements.

The bleaching of β-carotene is an indicator of its oxidation. Free radicals generated during linoleic acid peroxidation in an emulsion attack the β-carotene molecule and damage its double bonds; as a result, β-carotene undergoes rapid discoloration. The corresponding decrease in absorbance can be inspected spectrophotometrically. The presence of an antioxidant in the reaction mixture can limit the degree of β-carotene bleaching by neutralizing free radicals formed in the system [41]. According to Table 3 the antioxidant activities of OFI and OS extract increased with their increasing concentration. Again, the OS extract was more effective as antioxidant (EC_{50} = 2.93 ± 0.77 mg/ml) than the OFI one (EC_{50} = 4 ± 1.04 mg/ml). However the protection of the β-carotene bleaching by the samples was lower than that provided by the BHT standard (EC_{50} = 2.71 ± 0.05 mg/ml; Table 3).

4. Conclusion

The better results found for antioxidant activity of OSE through the above tests emphasize the idea that phenolic compounds can have a significant contribution to the prickly pears' antioxidant activity. Further, the results of the present study demonstrated that *O. streptacantha* is one of the *Opuntia* species that merits more investigation and research interest in order to unveil its advantages and develop its applications.

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