Scholars Academic Journal of Biosciences
Abbreviated Key Title: Sch Acad J Biosci
ISSN 2347-9515 (Print) | ISSN 2321-6883 (Online)
Journal homepage: https://saspublishers.com/journal/sajb/home

OPEN ACCESS

Biology

Evaluation of Antioxidant Activity and Polyphenols Contents of Extracts from Flowers of *Opuntia ficus*- indica L

Merghem Mounira*, Dahamna Saliha

Laboratory of Phytotherapy Applied to Chronic Diseases, Department of biology and animal physiology, Faculty of Natural and Life Sciences, Ferhat Abbas University, Sétif 19000, Algeria

***Corresponding author:** Merghem Mounira
| **Received:** 01.03.2019 | **Accepted:** 08.03.2019 | **Published:** 14.03.2019
DOI: 10.36347/sajb.2019.v07i03.003

Abstract | **Original Research Article**

Context: Opuntia ficus- indica L. a médicinal plant traditionally used in the treatement of liver disorders, rheumatism, renal diseases, hypoglycemic and hypocholesterolemic in Algeria. *Aims:* The aim of this study is to determine the content of polyphénols and flavonoïds and to evaluate the antioxidant activity of the aqueous (AqE) and methanolic (ME) extracts of flowers of *Opuntia ficus- indica* L. *Methods:* the content of polyphénols was determined by Folin-ciocalteu and flavonoids by Aluminium trichloride, Antioxidant activity *in vitro* was evaluated using DPPH (2'2-diphenylpicrylhydrazyl), β-carotene /linoleic acid bleaching, chelation of ferrous iron and reducing power tests. *Results:* The ME was richer in polyphénols (167, 1± 1, 74 µg AGE\mg extract) than the AqE (120, 9 ± 4, 24µg AGE\mg extract). The ME contains (56.07 ± 0, 16 µg QE\mg extract) of flavonoïds and the AqE contains (22.50 ± 0, 06 µg QE\mg extract). AqE has higher antiradical activity (IC_{50}=0,482 ± 0, 0228 mg/ml) than the ME (IC_{50}=3,813 ± 0,075 mg/ml). AqE and ME have a strong capacity to inhibit the lipid peroxidation in the β-carotene /linoleic acid bleaching test with an antioxidant activity of 89.791±0.015% and 83.079±0.074% respectively. The two studies extracts have a chelation activity, compared with the EDTA, the AqE has higher chelation activity (inhibition of the formation of Fe^{2+}-ferrosine complex) with IC_{50} = 4, 62 ± 0, 12 µg\ml than the ME with IC_{50} = 22, 64 ± 1, 53µg\ml. The ME has the higher reducing power (IC_{50}=0, 39± 0,005mg/ml) than AqE (IC_{50}=1, 22 ± 0,126 mg/ml). *Conclusions:* These results show the possibility of using the flowers of *Opuntia ficus- indica* L. as an antioxidant in folk medicine
Keywords: *Opuntia ficus-indica* L., polyphenols, flavonoïds, antioxidant activity.

INTRODUCTION

Opuntia ficus-indica L. commonly called prickly pear or nopal cactus, belongs to the dicotyledonous angiosperm *Cactaceae* family, a family that includes about 1500 species of cactus. *Opuntia ficus indica* is a tropical and subtropical plant [1]. this plant is widely distributed in arid and semi-arid regions of Africa, Central America, Mediterranean region and South Africa. Opuntia genus has more than 200 species [2].

Opuntia ficus-indica L. has been used in traditional folk medicine because of its role in treating a number of diseases, including antiinflammatory effects, hypoglycemic effects, inhibition of stomach ulceration, neuroprotective effects. Through antioxidant actions and also used for treating diabetes, burns, bronchial, asthma and indigestion in many countries over the world [3]. A flower infusion has an effect generally defined as depurative and in particular it is used because of its diuretic and relaxant action on the renal excretory tract [2].

The oxidative process is used for the production of energy required for essential cell activities; however, oxygen metabolism in the cells also causes the production of free radicals. Oxidizing agents are compound produced by metabolism, and if not controlled, can cause damage [4].

Recent studies have shown that a number of plant products including polyphenols, terpenes and various plant extracts exerted an antioxidant action. Antioxidants can be defined as substances that at low concentrations retard or prevent the oxidation of a substrate. They are responsible for the inhibition and reduction of lesions caused by free radicals in cells [4].

The main objectives of this study are the quantification of the phenolic compound, and the evaluation of the antioxidant activity of *Opuntia ficus-indica* flowers extracts.

MATERIALS AND METHODS

Plant material

Opuntia ficus-indica L. was collected from the Wilaya of Sétif in Northeast of Algeria; the flowers were cleaned with tap water, dried in the shade at room temperature for 2 weeks and ground into powder using an electric grinder.

Preparation of plant extract

Aqueous extract

50g of *Opuntia ficus-indica* L. flowers powder was mixed with 1L of boiling distilled water and after 20 minutes it was removed from the heat. The mixture was filtered using Wattman filter paper n°3 and then evaporated in rotary vacuum evaporator at 45°C.

Methanolic extract

The methanolic extract was obtained by maceration in water/methanol mixture (15:85) for 24 h. The resultant extract was filtered through Wattman paper n°3 and the solvent was removed by rotary evaporator under reduced pressure at 45°C. The resulting extract was then stored at -4°C until further analysis.

Determination of total polyphenol content

Total phenolic content was determined using Folin-Ciocalteu method, according to Li *et al.* [1] with slight modifications. A volume of 100 µl of the extract was mixed with 500 µl of Folin–Ciocalteau (diluted 10% in distilled water). After 4 min, 400 µl of sodium carbonate solution Na_2CO_3 (75 g/l) was added to the mixture, the reaction mixture was incubated at room temperature for 1h 30 min and the absorbance of the mixture was measured at 760 nm, Gallic acid (20-140 mg/l) was used as standard for the calibration curve. The total polyphenols content was expressed as micrograms of gallic acid equivalents (GAE) per milligram of extract. All samples were analyzed in three replications.

Determination of total flavonoids contents

The total flavonoids were determined using the aluminum trichloride ($AlCl_3$) method [6]. Briefly, 1 ml of 2% $AlCl_3$ in methanol was mixed with 1 ml of the extract. After incubation in dark at room temperature for 10 min, the absorbance of the reaction mixture was measured at 430 nm. Quercetin (1-40 mg/l) was used as standard for calibration curve and the total Flavonoids content was expressed as micrograms quercetin equivalent (QE) per milligram of extract.

Evaluation of antioxidant activity

DPPH free radical-scavenging assay

The free radical scavenging activity of the extracts was measured by 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay [7]. After dissolving the aqueous extract in distilled water, the methanolic extract in methanol, the solution of DPPH in methanol (0.04mg/ mL) was prepared and 1250 µL of this solution was added to 50µL of extracts solution at different concentration. The mixture was shaken vigorously and then kept in the dark for 30 minutes at room temperature. Then, the absorbance was measured at 517nm. rutin, quercetin and gallic acid were used as standards. All tests were performed in triplicate. Radical-scavenging activity was calculated using the following equation:

Radical scavenging activity
(%) = $(A_{blank} - A_{sample} / A_{blank}) \times 100$

A_{blank}: Absorbance of the control.

A_{sample}: Absorbance of the reagent with extract.

β-carotene/linoleic acid assay

In this test, the antioxidant capacity of the extracts was determined by measuring the inhibition of the oxidative degradation of β-carotene (discoloration or bleaching) by the oxidation products of the acid linoleic [8]. The β-carotene solution was prepared by dissolving 0.5 mg β-carotene in 1 mL of chloroform. One milliliter of this solution was pipetted to a flask covered with aluminum foil. Then 25 µL of linoleic acid and 200 mg of tween 40 were added. The chloroform was evaporated using evaporator at 45°C. Then 100 mL of distilled water saturated with oxygen was added. 2.5 mL of this prepared β-carotene solution were transferred to test tubes, and 350 µL of the extracts (2mg/mL methanol) were added before incubation for 48h at room temperature. The same procedure was repeated with butylated hydroxyl toluene (BHT) as a positive control and distilled water and methanol as a negative control. The absorbance was reading at 490 nm after 1h, 2h, 6h, 24h and 48h. The antioxidant activity of extracts was calculated using the following equation:

$AA\% = A_{sample} / A_{BHT} \times 100$.

A_{sample}: Absorbance in the presence of the Extract;
A_{BHT}: Absorbance in the presence of positive control BHT.

Chelation of ferrous iron

The chelating ability of the extracts is determined according to the method of Le *et al.* [9]. Which is based on the inhibition of the formation of Fe^{2+}-ferrosine complex after treatment of samples with Fe^{2+} ions. 250 µL of extract solutions at different concentrations were added to 50 µL of $Fecl_2$ (0.6 mM in distilled water) and 450 µL of methanol. After 5 min of incubation, 50 µL of ferrosine (5 mM in methanol) was added, and the mixture was stirred and allowed to react for 10 min to allow the complexation of residual iron. The absorbance of the Fe^{2+}-ferrosine complex was measured at 562 nm against a blank contained all the reaction reagents except ferrosine. Moreover, the negative control containing all reagents except that test

sample is replaced by the same volume of methanol. EDTA was used as reference chelator.

The chelating effect was calculated as a percentage, using the same equation as that in the DPPH assay.

Reducing power

Reducing power was determined on the basis of the ability of antioxidant principles to form colored complex with potassium ferricyanide, TCA and $FeCl_3$ and it was measured by the method reported by [10]. 1mL of extract was mixed with 0.5 mL phosphate buffer (0.2 M, pH 6.6) and 0.5 mL potassium ferricyanide (1% w/v). The mixture was incubated at 50°C for 20 min. After terminating the reaction by adding trichloroacetic acid (10% w/v), the mixture was centrifuged at 3000 rpm for 10 min. The supernatant of solution 0.5 mL was mixed with 0.5 mL distilled water and 0.1 mL FeCl3 (0.1% w/v). After 5 min later, the absorbance was measured at 700 nm. Higher absorbance of the reaction mixture indicated higher reducing power.

Statistical Analyses

The results are expressed as the mean ± standard deviation. One-way analysis of variance (ANOVA) followed by the Tukey test was performed to assess differences between groups. Differences were considered significant at $p < 0.05$.

RESULTS

Total phenolic and flavonoid content

The content of total phenolic compound is given in Table 1. It was noticed that the methanolic extract showed higher total polyphenolic content 167,1 ± 1,74 µg GAE/mg and flavonoids 56.07 ± 0,16 µg QE/mg than the aqueous extract 120,9 ± 4,24 µg GAE/mg and 22.50 ± 0,06 µg QE/mg respectively.

Table-1: Total polyphenols and flavonoids in *Opuntia ficus-indica* L. flowers extracts

Extract	Polyphenols µg GAE/mg extract	Flavonoids µg QE/mg extract
AqE	120,9 ± 4,24	22.50 ± 0,06
ME	167,1 ± 1,74	56.07 ± 0,16

ME; methanolic extract, AqE; aqueous extract; GAE: gallic acid equivalent; QE: quercetin equivalent .Each value represents the mean ± SD (n = 3).

Antioxidant activity evaluation

DPPH radical scavenging activity

Results of DPPH scavenging activity of *Opuntia ficus-indica* L. flowers extracts are given in Table 2. AqE exhibited the highest activity toward DPPH scavenging (IC_{50} = 0,482 ± 0,0228 mg/ml) followed by ME with (IC_{50} = 3,813 ± 0,075 mg/ml).

Table-2: DPPH scavenging activity of *Opuntia ficus-indica* L. flowers extracts and standards

Extracts	IC_{50} (mg /ml)
ME	3,813 ± 0,075
AqE	0,482 ± 0,0228
Gallic acid	0,056 ±0,001#
Quercetin	3,491 ± 0,001#
Rutin	4,179 ± 0,000#

#: µg/ml. Each value represents the mean ± SD (n = 3).

β-carotene/linoleic acid bleaching assay

The Results are presented in Table 3. AqE and ME exhibited the highest antioxidant activity (89,79% and 83,079%, respectively), which was near to that of BHT (100%).

Table-3: Antioxidant activities of *Opuntia ficus-indica* L. flowers extracts at 24 hours of incubation measured by β-carotene bleaching method.

Extracts	Inhibition %
ME	83,079±0.074
AqE	89,791±0.015
BHT	100±0.001
MeOH	15,99±0.12
H_2O	10,603±0.34

Each value represents the mean ± SD (n = 3).

Metal chelating activity

The results are shown in table 4. The AqE appear to have the important chelating activity with IC_{50} values of 4,62 ± 0,12 µg/ml than ME 22,64 ± 1,53 µg/ml, respectively.

Table-4: Metal chelating activity of *Opuntia ficus-indica* L. flowers extracts and EDTA.

Sample	IC_{50} (µg/ml)
ME	22,64 ± 1,53***
AqE	4,62 ± 0,12 ^{ns}
EDTA	5,05 ± 0,09

Comparison was realized against EDTA, ns: not significant, ***: $p \leq 0.001$. Each value represents the mean ± SD (n = 3).

Reducing power

The results obtained (table 5) showed that the ME has the highest reducing power (IC_{50} = 0.39 ± 0.005 mg / ml) than the AqE (IC_{50} = 1.23 ± 0.13 mg / ml). Both extracts have a reducing power lower than of BHT (IC_{50} = 0.05 ± 0.006 mg / ml).

Table-5 : IC_{50} values of *Opuntia ficus-indica* L. flowers extracts for reducing power test

Sample	*IC_{50} (mg /ml)*
AqE	*1,22 ± 0,126 ******
ME	*0,39 ± 0,005***
BHT	*0,05 ± 0,006*

**: $p \leq 0.01$, ***: $p \leq 0.001$. Each value represents the mean ± SD (n = 3).

DISCUSSION

Plant phenolics, tannins and flavonoids represent major groups of plant constituents that work predominantly as powerful antioxidants or scavenger of free radicals. They play beneficial role in human health and cure or prevent ailments such as inflammatory disorders, cardiovascular diseases, cancer and diabetes which occur due to the deregulation of free radicals generation in the cells [11].

The quantity of the phenolic compounds of the studied plants depends mainly on their origin [12], variety, the season of culture, the season of harvest, the climatic conditions and environmental, the geographical location, the various diseases which can affect the plant, the maturity of the plant [13].

According to Chu *et al*. [14], total antioxidant activities of the plant extracts cannot be evaluated with a single method due to the complex nature of phytochemicals. Two or more methods should always be employed in order to evaluate the total antioxidative effects of plant material [15]. Thus, in the present study we applied four methods *in vitro*, DPPH scavenging activity, β-carotene bleaching, ferrous ion chelating and reducing power to evaluate the antioxidant capacities of *Opuntia ficus-indica* L. flowers extracts.

DPPH radical is a stable free radical that shows a maximum absorption at 517 nm, and is widely used to evaluate the free radical scavenging ability of natural compounds. In the DPPH assay, the antioxidants were able to reduce the stable radical DPPH (purple color) to the yellow coloured diphenylpicrylhydrazine. Therefore, the antioxidant activities of a sample can be expressed as its ability in scavenging the DPPH radical [16].

The anti-radical activity may be related to the presence of flavonoids and polyphenols in the extracts. The antioxidant effect of plant extract is likely related to the amount of polyphenols present [10, 17], and may also differ depending on the quality of polyphenols and flavonoids [18]. The mechanism of the reaction between the antioxidants and DPPH depends on the structural conformation of the antioxidant. Some compounds react rapidly with the DPPH, with the reducing the number of DPPH equal to that of the hydroxyl groups present in the antioxidant compound [19].

The bleaching of β-carotene is a free radical-mediated phenomenon resulting from the hydroperoxides formed from linoleic acid. In this model system, β-carotene undergoes rapid discoloration in the absence of an antioxidant. This is because of the coupled oxidation of β-carotene and linoleic acid, which generates free radicals. The linoleic acid free radicals, formed upon the abstraction of a hydrogen atom from one of its diallylic methylene groups, attacks the highly unsaturated β-carotene molecules. As a result, β-carotene will be oxidized and broken down in part; subsequently, the system loses its chromophore and characteristic orange color, which can be monitored spectrophotometrically. The presence of different antioxidants can hinder the extent of β-carotene bleaching by neutralizing the linoleic acid-free radical and other free radicals formed in the system [10].

The antioxidant potentials of the extracts were evaluated by determining their ferrous ion chelating ability. ferrous ion chelating ability measures the ability of secondary antioxidants to chelate metal ions. Primary antioxidants prevent oxidative damage by directly scavenging free radicals, while secondary antioxidants act indirectly by preventing the formation of free radicals

through Fenton's reaction [14]. Fe^{2+} has been known to accelerate formation of hydroxyl radicals via the Fenton reaction, leading to occurrence of many diseases. It was reported that chelating agents, which form σ-bonds with a metal, are effective as secondary antioxidants because they reduce the redox potential, thereby stabilizing the oxidized form of the metal ion [20]. In the Ferrous ion chelating assay, ferrozine acts as a chelating agent and forms purple complex ion with Fe^{2+} giving maximum absorbance at 562 nm. The complex formation can be disrupted by the presence of other complexing agents which cause a decrease in the purple color intensity of complexes. Substances or samples that can reduce its color intensity can be considered as antioxidant through the mechanism of inhibition of heavy metal [21].

Studies have shown that *Opuntia* was rich in natural antioxidant compounds such as carbohydrates, flavonoids, mineral amino acids (arginine), phenolic acids (caffeic and gallic), polyphenols, quercetin, tannins, tocopherol, and sulfur amino acids (cysteine, methionine, and taurine). Flavonoids of cladodes possessed many notable biological activities; such as inhibition of lipid peroxidation, oxidation of low density lipoproteins, anti-inflammatory, anti-carcinogenic, anti-aggregatory, antioxidant, hypoglycemic and antiviral activities [22].

All parts of the *Opuntia ficus-indica* L. are rich in members of the polyphenol family such as various flavonoids and phenolic acids. In the flower, gallic acid and 6-isorhamnetin 3-*O*-robinobioside are the major compounds, amounting to 4900 and 4269 mg/100 g of dry matter, respectively. Other phenolic molecules are present in small quantities not exceeding 10 mg/g [23].

Health beneficial effects of *Opuntia ficus-indica* L. polyphenols might be conditioned by their antioxidant and radical scavenging activities. For instance, gallic acid, largely found in flowers, exhibits high antioxidant activity responsible for its ability to reduce DNA damage and to buffer free radicals. At a concentration of 4.17 mM, it may neutralize 44% of 2,2-diphenyl-1-picrylhydrazyl radical and 60% of hydrogen peroxide in given experimental conditions. Gallic acid also exerts a cytotoxic activity against tumoral cells from leukemia, lung and prostate cancer origins [23].

CONCLUSION

The tested *Opuntia ficus-indica* flowers extracts were shown to be having high phenolic content and potent antioxidant activities achieved by DPPH (2'2-diphenylpicrylhydrazyl), β-carotene /linoleic acid bleaching, chelation of ferrous iron and reducing power tests.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

This work was supported by the Algerian Ministry of Higher Education and Scientific Research (MERS) and by the Algerian Agency for the Development of Research in Health (ANDRS). We express our gratitude to these organizations.

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