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ANTIOXIDANT ACTIVITY OF *OPUNTIA FICUS-INDICA* FLOWERS PHENOLIC EXTRACTS

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ABSTRACT: The cactus plant *Oputia ficus-indica*, is a member of the Cactaceae family, native to Mexico, mainly cultivated for its cladodes, flowers, and fruits. Opuntia flower is useful in numerous fields; such as traditional medicines. Phenolic Flowers extracts of *opuntia ficus-indica* were phytochemical studied and in-vitro tested for their potential antioxydant activity using four methods, the total antioxidant capacity, reducing power, DPPH radical scavenging activity, and β-carotene bleaching assay. The results showed the flowers richness by phenolic compounds and flavonoids (120.22mgGAE/gDW, 62.67mgGAE/gDW) and the different levels of high antioxidant properties for all extracts specially for the flavonoid ethyl acetate extract which showed the highest activity to reducing power, to scavenge DPPH radical with an IC_{50} of 0.27 $mg.mL^{-1}$ and to inhibit the oxidation of β-carotene with an IC_{50} of 0.39 $mg\ mL^{-1}$, the latter is better than the capacity to inhibiting the oxidation of β-carotene of ascorbic acid whose IC_{50} = 0.43 $mgmL^{-1}$. Hence, the Opuntia flowers provide a source of natural antioxidant, suggesting that it may be considered as a potential candidate of possible health-promoting functional foods.

INTRODUCTION: There is increasing evidence that fruits and vegetables may protect against numerous chronic diseases, including cancer, cardio- and cerebrovascular, ocular, and neurological diseases [1, 2, 3, 4]. The protective effect of vegetables has generally been attributed to their antioxidant constituents, including vitamin C (ascorbic acid), vitamin E (α-tocopherol), carotenoids, glutathione, flavonoids, and phenolic acid, as well as other unidentified compounds [5].

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Polyphenolic flavonoids are metabolic products widely distributed in foods of plant origin, and they have numerous biological and pharmacological properties [6, 7] that could potentially afford protection against chronic diseases. Natural foods have recently received immense attention from health professionals as well as the consumers in the wake of the discovery of their health-promoting potential. In this context, Cactus have emerged as promising candidates [8].

The cactus plant *Opuntia* spp., a member of Cactaceae family, is widely spread in Algeria arid and semi-arid region, it develops on the Mediterranean shore and particularly in Kabylie [9]. Different parts of *O. ficus-indica* are used in the traditional medicine of several countries: Many uses of cactus pear fruit and cladodes are reported

[10], the cladodes are utilized to reduce serum cholesterol level and blood pressure, for treatment of ulcers, rheumatic pain, wounds, fatigue, capillary fragility, and liver conditions [11]. Cladodes are also highly used for the food industry; they have been studied for their gelling properties [12]. The fruits are rich in nutritional compounds, such as ascorbic acid and polyphenols. These fruits have shown antiulcerogenic [13], antioxidant [13, 14, 15], anticancer [16], neuroprotective [17], hepatoprotective [18], and antiproliferative activity [19]. The flower was little studied because of their limited bloom duration and in addition to that the difficulty to get them.

Opuntia flower is useful in traditional medicines thanks to its effect which are defined as depurative and in particularly diuretic and relaxant of renal excretory tract [20]. Many kinds of flavonoids have been reported in *Opuntia* cactus, and types and content vary with variety, tissue type, and maturation [21]. In the literature, few reports are focused on *Opuntia* flowers. Bergaoui *et al.* [22] have reported the chemical composition of volatiles fractions from aqueous distillate obtained from three *Opuntia* species (*Opuntia lindheimeri*, *Opuntia macrohiza*, and *Opuntia microdasys*). The recent study of De Léo *et al.* [23] describes the chemical content of methanol extract *O. ficus-indica* flowers. Therefore, to provide major information about the phytochemical composition of *O. ficus-indica* flowers, we performed the analysis of phenolic extracts of plant material from Algeria. The aim of the present work was to evaluate the polyphenol composition and antioxidant activity of the phenolic extracts isolated from *Opuntia* flowers.

MATERIALS AND METHODS:

Plant Material: *Opuntia ficus-indica* inermis flowers were harvested in the spring season of 2012 in the region of AIN YOUCEF, Tlemcen northwest of Algeria. In the laboratory, flowers were dried under shade and grounded with Moulinex blinder, then stored in glass vials protected from light at -20 °C which were used for extractions. All analyses were performed in duplicate.

Plants were identified at the laboratory of Ecology and Management of Natural Ecosystems of the University of Tlemcen (Algeria).

Extraction of Chemical Compounds from Opuntia Flowers:

Total Phenolic Compounds: The dried flowers (10 g) were ground and extracted with acetone – water (70/30, v/v) by maceration at room temperature for 24 h [24]. The filtrate was concentrated to dryness under reduced pressure at 45 °C and was stored at 4 °C, for further investigation.

Total Flavonoids: 10 g of dried material were extracted with 100 ml of methanol (MeOH) and 5g of carbonate of Calcium by boiling for 1 h [25]. After filtration, the MeOH was evaporated under reduced pressure. Subsequently, recover the dry extract with 50 ml of boiling water. The aqueous extract was filtered and then fractionated by (solvent – solvent) extraction, first with Diethyl Ether, Ethyl acetate and then with n-butanol, using a separating funnel (Pyrex). All the fractions were concentrated and kept at 4 °C.

Tannins: Tannins extraction from *Opuntia ficus-indica* flowers (5 g) was extracted at 4°C using 200 ml of a mixture of acetone – water (25/45, v/v) for 4 days [26]. The extracts were filtered under vacuum through filter paper, and acetone was evaporated under reduced pressure. Subsequently, the dichloromethane (2 × 25 ml) was used for the extraction of lipids and pigments from the aqueous extracts using a separating funnel. Afterward, the aqueous phase was extracted with 25 ml of ethyl acetate. This process was repeated 4 times. After filtration, the organic phases (ethyl acetate) containing tannins were recovered and concentrated to dryness under vacuum using a rotary evaporator. The residues obtained were weighed and preserved until uses.

Determination of Total Phenolics Contents and Tannins: Total phenolics contents of samples obtained from flowers phenolic extracts was determined using the Folin–Ciocalteu reagent according to the modified method of Singleton and Rossi [27] with gallic acid as standard. The amount of total phenolics compounds was calculated as mg of gallic acid equivalents (GAE) and expressed as mg gallic acid/g dry weight (DW) of the plant material. The calibration equation for gallic acid was;

$$y = 3.180x - 0.030 \; ; (R^2 = 0.991)$$

Where y is the absorbance and x is the concentration of gallic acid in mg/ml.

Using the same extract, the tannins were estimated after treatment with polyvinyl polypyrrolidine (PVPP) [28]. One-hundred mg of PVPP was weighed into a 100 × 12 mm

Eppendorf tube and to this 1 mL distilled water and then 1 ml of the sample extracts were added. The content was vortexed and kept in the freezer at 4 ºC for 15 min. Then the sample was centrifuged at 1.681×g for 10 min at room temperature, and the supernatant was collected. This supernatant has only simple phenolics other than the tannins (the tannins would have been precipitated along with the PVPP). The phenolic content of the supernatant was measured and expressed as the content of non-tannin phenolics on a dry matter basis. From the above results, the tannin content of the sample was calculated as follows:

Tannins (%) = Total phenolics (%) − Non-tannin phenolics (%)

Determination of Total Flavonoids Contents: Total flavonoid content was determined according to Djeridane *et al.,* [29] using a method based on the formation of a flavonoid–aluminum complex, having the maximum absorbance at 430 nm. 1 mL of diluted sample (1mg/mL) was mixed with 1 mL of 2% aluminum trichloride ($AlCl_3$) methanolic solution. After incubation at room temperature for 15 min, the absorbance of the reaction mixture was measured at 430 nm with an Analytik jena, SPEC ORD 200 PLUS, spectrophotometer.

The concentrations of flavonoid compound were calculated according to the equation obtained from the standard Catechine graph.

The total flavonoid content was expressed as milligram of Catechine equivalent (CE) per gram of extract.

***In-vitro* Antioxidant Activity Determination:**

Total Antioxidant Capacity by Phospho-molybdate Method: The total antioxidant capacity of the plant extract/fraction was evaluated by measuring the absorbance of Mo(V) at 695nm [30]. An aliquot of 0.2 ml of the sample solution was mixed with 2.0 ml of the reagent solution (600mM sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The reaction mixture was incubated at 95 °C for 60 min, and absorbance was measured at 695 nm against a blank containing 2 mL of reagent solution. Total antioxidant capacity was expressed equivalent to ascorbic acid.

DPPH Radical Scavenging Assay: DPPH radical scavenging ability was measured using the method of Brand-Williams *et al.* [31] Sample (0.2 mL) was mixed with 2.8 mL DPPH solution (60 µmol·L^{-1}), and the mixture was allowed to stand for 30 min in the dark at room temperature. Absorbance was measured at 515 nm using a spectrophotometer. Trolox and Ascorbic acid was used as a comparison. Scavenging ability was calculated using the following formula:

Scavenging ability (%) = [(Absorbance 515 nm of control – Absorbance 515 nm of sample) / Absorbance 515 nm of control] × 100

Determination of Reducing Power: The reducing power assay was determined according to the method of Oyaizu [32] with little modification. The tested samples (0.1 ml) were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide (2.5ml, 1%). After the mixture was incubated at 50 °C for 20 min. Trichloracetic acid (2.5 mL, 10%) was added to each sample and centrifuged at 3000 rpm for 10 min.

A 5 ml aliquot of the upper layer was mixed with distilled water (5 ml), and ferric chlorid (1 ml, 0.1%) was added, and then the absorbance was measured at 700 nm against a blank which consists of all the reagents without the tested sample. The higher absorbance indicated higher reducing power. The reducing power of Ascorbic acid and Trolox were also determined for comparison.

β-carotene Bleaching Inhibition Capacity Assay: The antioxidant activity of methanolic extracts was evaluated using a β-carotene-linoleate model system, as described by Moure *et al.* [33] Two milligrams of β-carotene were dissolved in 10 mL chloroform, and 1 mL of β-carotene solution was mixed with 20 µL of purified linoleic acid and 200 mg of Tween 40 emulsifier. Chloroform was removed in a rotary vacuum evaporator, and the resulting mixture was immediately diluted with 100 mL of distilled water. To an aliquot of 4 mL of this emulsion, 200 µL of the methanolic solution of

extracts or the reference antioxidants (Gallic acid and BHA) were added and mixed well.

The absorbance at 470 nm, which was considered at t = 0 min, was immediately measured against a blank, consisting of the emulsion without β-carotene. The capped tubes were placed in a water bath at 50 °C for a period of 2 h. After that, the absorbance of each sample was measured at 470 nm (A120). BHA was used for positive control. The negative control consisted of 200 µL methanol instead of methanolic extract or BHA. All tests were repeated twice. The antioxidant activity (AA) was calculated according to the following equation:

$$AA = [(AA(120) – AC(120))/ (AC(0) – AC(120)] \times 100$$

Where AA(120) is the absorbance of the sample at t = 120 min; AC(120) the absorbance of the control at t = 120 min and AC(0) the absorbance of the control at t= 0 min.

Statistical Analysis: All evaluations of dosage and antioxidant activity were performed in triplicate. Data were expressed as mean ± standard derivation (SD).

RESULTS AND DISCUSSIONS:

Total Phenolics Content, Total Flavonoids, and Tannins of *Opuntia ficus-indica* Flowers: Flavonoids and phenolic compounds are well known as antioxidants. In various studies, the antioxidant activity of plant extracts was found to be fairly high when they are rich in phenolic compounds [34]. This high potential of phenolic compounds to scavenge radicals may be explained by their phenolic hydroxyl groups [35]. For that, total polyphenols, flavonoids, and tannins were assessed in phenolic flowers extract. Results have demonstrated the wealth of *Opuntia* flowers in phenolic compounds 120.22 ± 1.00 mg GAE/g DW followed by a high level of flavonoids 62.67± 0.15 mg CE/g DW and finally less rich in tannins 7.6867± 0.05mgGAE/g DW **Table 1**.

Compared with cladodes of *Opuntia ficus-indica*, these values were higher than those reported by Dib *et al.* [36] The study made by Alimi *et al.,* [37] about phytochemical content of *Opuntia ficus-indica* flowers extracted with 50% methanol solution has demonstrated a content greater than ours of total phenolic content (159.76 ± 0.32 mgGAE / g of extract) also a higher flavonoid (79.51 ± 0.57 mgRE/g of extract). This is why methanol is often used for extraction of medium polar and polar phenolic compounds such as flavonoid glycosides and phenolic acids [38]. This richness of flowers of *Opuntia ficus-indica* in bioactive compounds suggests it as a good source of all investigated phytochemically.

Antioxidant Activity Evaluation: To the best of our knowledge, there are no reports in the literature concerning the antioxidant activity of *O. ficus-indica* flowers. The bioactive compound of flowers was subjected to screening for their possible antioxidant activities.

TABLE 1: TOTAL PHENOLIC, FLAVONOID AND TANNINS CONTENT IN FLOWERS OF *OPUNTIA FICUS-INDICA*

Chemical constituent	Total phenolic Contents (mgGAE/gDW)	Total flavonoids content (mgQE/gDW)	Total Tannins Content (mgGAE/gDW)
Flowers	120.22 ± 1.004	62.67 ± 0.15	7.6867 ± 0.05

The data are displayed with a mean ± standard deviation of triplicate. Mean values followed by different superscript in a column are significantly different ($p<0.05$).

Owing to the complex reactive facets of phytochemicals, the antioxidant activities of plant extracts cannot be evaluated by only a single method, but atleast two test systems have been recommended for the determination of antioxidant activity to establish authenticity [39].

For this reason, the antioxidant activity of various extracts of flowers of *O. ficus-indica* was determined by four spectrophotometric methods, total antioxidant capacity, scavenging activity of DPPH radical, reducing power assay and β-caroten/linoleic acid assay methods, at different concentrations**.**

Total Antioxidant Capacity (TAC): Total antioxidant capacity of *Opuntia* flowers phenolic extracts, expressed in equivalents of ascorbic acid. The extracts showed electron-donating capacity and thus they may act as radical chain terminators, transforming reactive free radical species into more stable non-reactive products [40].

The extracts exhibited some degree of activity in a dose-dependent manner. The study revealed that all extracts have antioxidant capacity. The antioxidant activity of polyphenols was similar to that of tannins, ditto for flavonoid extracts (ethyl ether and n-butanol) from flowers of *Opuntia* **Table 2**. Moreover ethyl acetate flavonoids extract have stronger antioxidant activity (0.406 ± 0.05 mgAEE/g extract) followed by tannins (0.14 ± 0.005 mgAEE/g extract) and polyphenols (0.121 ± 0.004 mgAEE/g extract). Flavonoids extracts have antioxidant activity which is classified as follows: acetate ethyl, n-butanol, and ethyl ether. We note that the ethyl acetate extracted from flowers of *Opuntia ficus indica* showed antioxidant capacity of most interesting to the reduction of Mo (VI) Mo (V), so they are quantitatively and qualitatively high effective on phospholybdique test, its confirmed by many recent studies which show that many flavonoids and related polyphenols contribute significantly to the total antioxidant activity of many fruits such as the red grape [41], vegetables [42] and medicinal plants [43].

TABLE 2: TOTAL ANTIOXIDANT CAPACITY IN *OPUNTIA FICUS-INDICA* FLOWERS EXTRACT

Bioactive Compunds	TAC (mgAAE/gDW)
Polyphenols	0.12 ± 0.004
Flavonoids (ethyl ether)	0.02 ± 0.005
Flavonoids (ethyl acetate)	0.406 ± 0.05
Flavonoids (n-butanol)	0.07 ± 0.002
Tannins	0.14 ± 0.005

The data are displayed with a mean ± standard deviation of triplicate. Mean values followed by different superscript in a column are significantly different ($p<0.05$).

Determination of the Scavenging Effect on DPPH• Radicals: Free radical assay is one of the most widely used methods in establishing the antioxidant activity of herbal extracts and photochemical. DPPH is known to abstract labile hydrogen, and the ability to scavenge the DPPH radical is related to the inhibition of lipid peroxidation [44]. The scavenging activity was expressed by the percentage of DPPH reduction after 30min of reaction. The measurements were triplicate, and their scavenging effects were calculated based on the percentage of DPPH scavenged [45, 46].

Besides, the antioxidant potential of the phenolic compounds depends on the number and the arrangements of hydroxyls groups as well of the presence of constituents' donors of electrons [47]. IC_{50} values were found to be the least in ethyl acetate flavonoid extract IC_{50} of 0.27±0.007 mg/ml, followed by n-butanol flavonoids extract IC_{50} of 0.45 ± 0.01 mg/ml, ethyl ether flavonoids extract IC_{50} of 0.999 ± 0.011 mg/ml, tannins IC_{50} of 1. 50 ± 0.09 mg/ml and polyphenols IC_{50} of 2.64 ± 0.18 mg/ml **Table 3**. However, DPPH free radical scavenging of all the secondary metabolites tested was lower than that of ascorbic acid and Trolox (0.12 and 0.15 mg mL^{-1}). There is a lack of information available on the chemical composition of flowers from *Opuntia ficus-indica* inducing antioxidant activity.

Further phytochemical investigations on these extracts, including fractionation, are needed to isolate active constituents and subsequent pharmacological evaluation.

TABLE 3: DPPH IC_{50} mg/ml VALUES OF DIFFERENT EXTRACTS OF *OPUNTIA FICUS-INDICA* FLOWER

Bioactive compounds	DPPH IC_{50} (mg/ml)
Polyphenols	2.64 ± 0.18
Flavonoids ethyl acetate	0.27 ± 0.007
Flavonoids n-butanol	0.45 ± 0.01
Flavonoids ethyl ether	0.999 ± .011
Tannins	1.50 ± 0.09
Ascorbic acid	0.12 ± 0.08
Trolox	0.152 ± 0.049

The data are displayed with a mean ± standard deviation of triplicate. Mean values followed by different superscript in a column are significantly different ($p<0.05$).

Ferric Reducing Antioxidant Power Assay (FRAP): Tanaka *et al.*, observed a direct correlation between antioxidant activities and reducing the power of certain plant extracts [48]. The reducing properties are generally associated with the presence of reductones [49], which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom [50].

In this assay, depending on the reducing power of antioxidant compounds, the yellow color of the test solution changes into various shades of green and blue. Therefore, by measuring the formation of Perl's Prussian blue at 700 nm, we can monitor the Fe^{2+} concentration.

Reducing the power of different extracts (phenolic compounds, flavonoids, and tannins) of *Opuntia ficus-indica* flowers and standards (Ascorbic acid and TROLOX) using the potassium ferricyanide reduction method were described in **Fig. 1**. All phenolic extract of *Opuntia* flowers have indicated a good reducing power but lower than that of the ascorbic acid and Trolox. Unlike for ethyl acetate flavonoid extract which demonstrated the greatest reducing power, better than standards.

The high reducing effect of flowers extracts might be attributed to the presence of phytochemicals such as phenolic compounds [51], flavonoids and tannins presented in our previous results, with high contents and this is confirmed with the study conducted by Ghalem *et al.* [52] which evaluated the ferric reducing antioxidant power of *Anthyllis vulneraria* L. flowers.

They concluded that polyphenols of *A. vulneraria* flowers had the highest value of FRAP while the flavonoids butanol extract was found to be less significant. Moreover, polyphenols and tannins extract were significantly more pronounced than that of Ascorbic acid.

Due to the high total phenolic content of sample extract, these phenolic compounds represent the primary source of this antioxidant activity.

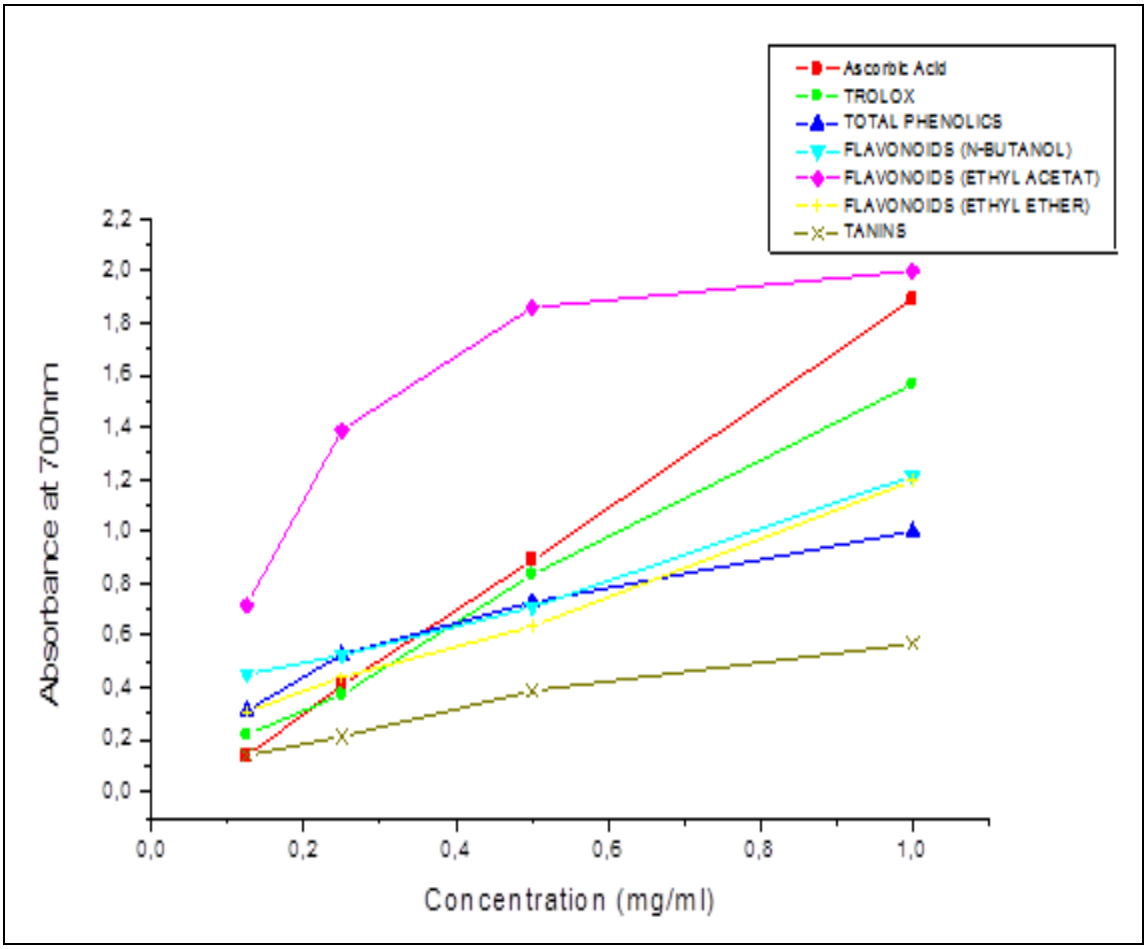

FIG. 1: TOTAL REDUCING POWER OF DIFFERENT EXTRACTS (POLYPHENOLS COMPOUND, FLAVONOIDS, AND TOTAL TANNINS) OF *OPUNTIA FICUS-INDICA* FLOWERS

β-carotene Bleaching Inhibition Capacity Assay: In this model system, *β*-carotene undergoes rapid discoloration in the absence of an antioxidant. This is due to the oxidation of linoleic acid that generates free radicals that attacks the highly unsaturated *β*-carotene molecules. The presence of antioxidant avoids the destruction of the *β*-carotene conjugate system, and the orange color is maintained [53]. The obtained results are summarized in **Table 4**.

The results show that all the extracts were capable of inhibiting the bleaching of *β*-carotene by scavenging linoleate-derived free radicals and were more effective in comparison with gallic acid. Most effective was ethyl acetate flavonoid extract (0. 39 ± 0.016 mg/ml) and tannins extract (0, 41 ± 0,051 mg/ml) of *Opuntia ficus-indica* flowers and were similar to high than gallic acid (0.43 ± 0.008 mg/ml) but still lower than Trolox (0.242 ± 0.002 mg/ml).

TABLE 4: **β-Carotene IC_{50} (mg/ml) VALUES OF DIFFERENT EXTRACTS OF *OPUNTIA FICUS-INDICA* FLOWERS**

Bioactive Compounds	β-carotene IC_{50} (mg/ml)
Polyphenols	1.04 ±0.05
Flavonoids ethyl acetate	0.39 ±0.016
Flavonoids n-butanol	1.26 ±0.045
Flavonoids ethyl ether	4.42 ±0.04
Tannins	0.41 ±0.051
Gallic acid	0.43 ±0.008
Trolox	0.242±0.0028

The data are displayed with a mean ± standard deviation of triplicate. Mean values followed by different superscript in a column are significantly different ($p<0.05$).

CONCLUSION: The results present in this study are the first information on the chemical composition and antioxidant activities of *Opuntia* flowers. The phenolic extracts of *O. ficus-indica* flowers contained a high level of total phenolic and flavonoid compounds and were capable of inhibiting radicals and acting as reducing agents. Accordingly, in this study, a significant and linear relationship was found between the antioxidant activity and phenolic content, indicating that phenolic compounds could be major contributors to antioxidant activity.

As for the chemical composition, amounts and nature of compounds vary with flowering stages and species, suggesting changes in secondary metabolism of flowers [20].

This richness of *Opuntia* flowers in interesting compounds can support the utilization of these flowers in various fields of application including agro-alimentary, cosmetic, and pharmaceutical.

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CONFLICT OF INTEREST: Nil

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