J. Phytomol. Pharmacol., 4(1), 01-06, 2025
https://doi.org/10.56717/jpp.2025.v04i01.032
ISSN(Print): 2957-5818, ISSN(Online): 2958-6224

Journal of Phytomolecules & Pharmacology

Research Article

A comparative study of total polyphenolic content and antioxidant activity of prickly Pear (*Opuntia ficus-indica* L.) seeds extract

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Abstract

Opuntia ficus-indica fruits have been widely used due to their nutritional composition and beneficial effects on human health. In recent years, researchers have focused on ~~the~~ *Opuntia* seeds, which contain bioactive molecules with antioxidant~~s~~ properties. In this context, the objective of this study is to demonstrate the richness of *Opuntia ficus-indica* seeds collected from three cultivated populations in Tunisia (Kasserine, Gafsa, and Sidi Bouzid) by ~~estimation of~~ estimating total polyphenolic and flavonoid contents (TPC and TFC) and evaluation of their antioxidant potential. The TPC of *Opuntia* seeds hydroethanolic extract of three regions ranged from 5.37 mg to 6.92 mg gallic acid equivalent/g dry extract (mg GAE/g DE). The TFC varied from 2.16 mg to 2.85 mg quercetin equivalent/g dry extract (mg QE/g DE). It can be inferred that the population of Kasserine is the richest in antioxidants with a value of 4.97 mg ascorbic acid equivalent/g dry extract (mg AAE/ g DE), followed by the population of Sidi Bouzid with a value of 4.22 mg AAE/ g DE and 3.55 mg AAE/ g DE for the population of Gafsa. These results confirm that *Opuncia* seeds have good potential as antioxidant and can be useful in the pharmaceutical, cosmetics, and food industries with appreciable human health-promoting properties.

Article Information
Received: 16 February 2025
Revised: 22 February 2025
Accepted: 24 February 2025
Published: 05 March 2025

Academic Editor
Prof. Dr. Marcello Iriti

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Keywords
Opuntia ficus-indica L., total polyphenolic content, total flavonoid content, antioxidant potential.

1. Introduction

Aromatic and medicinal plants have been used for thousands of years as natural medicines, due to their rich bioactive compounds [1]. Among the sources of these bioactive molecules, the *Opuntia* genus is particularly noted for its long history of use for different food, pharmaceutical and medicinal purposes, which could be attributed to its polyphenolic compounds [2, 3]. *Opuntia* spp. belongs to the Cactaceae family, native to the American continent, comprises more than 300 species and grows in very adverse conditions, making it especially interesting for cultivation in arid regions around the World [4]. Among these species, prickly pear (*Opuntia ficus-indica*), is of great interest for its richness in bioactive compounds and for its various therapeutic activities, including antioxidant, antimicrobial and

anti-inflammatory activity [5-7]. It is commonly cultivated for its fruits, used as food, natural dye, sweetener and fodder, and is also recognized for its medicinal properties, including as an antidiabetic in traditional medicine [8-10].

Opuntia ficus-indica fruits have been widely used due to their nutritional composition and beneficial effects on health, particularly against chronic diseases such as diabetes, obesity, cardiovascular diseases and cancer, among others [11]. The beneficial health effects of these fruits are mainly related to the presence of polyphenolic compounds [12-14]. Phytochemical analyses of *Opuntia* fruit extracts reveal high levels of bioactive compounds, including polyphenols, vitamins C and E, β-carotene, glutathione and a combination of betaxanthin and betacyanin pigments. These bioactive molecules act as powerful antioxidants, effectively fighting free radicals and mitigating oxidative stress [15]. Indeed, these compounds have been studied for their biological properties with beneficial properties for human health and could be useful to replace or even decrease synthetic antioxidants in food, cosmetics and pharmaceutical industries.

In this context, our study has been undertaken to estimate the total polyphenolic content, total flavonoid content, and the antioxidant activity of Tunisian cultivated prickly pear (*Opuntia ficus-indica)*, in order to promote this plant as a potential source of bioactive molecules with beneficial effects for human health and to increase the economic value of this rain fed crop for the rural development in this country.

2. Materials and methods

2.1. Plant material

Mature fruits of *Opuntia ficus-indica* were randomly collected from three Tunisian regions (Kasserine, Sidi-Bouzid and Gafsa). Voucher specimens of prickly pear from every location (OFIK23, OFIS-B23, and OFIG23) were identified by Dr. Sondes Stambouli-Essassi and deposited at the herbarium of the Faculty of Sciences of Gafsa. Details of collection sites are given in Table 1. Collected fruits were washed with water to remove dust and spines, peeled and blended by a Moulinex blender, and then the seeds were separated from the juice by passing the mixture through a sieve with a 2mm mesh size. Seeds were washed with distilled water, dried at room temperature for 15 days, afterwards dried in a forced-air drier at 35 °C for 48 h, and then ground to a fine powder using a Moulinex coffee grinder and stored in a smoked bottle at 4°C until use.

2.2. Preparation of Opuntia seeds extract

Dried samples (2g) were macerated in 20 ml of hydroethanolic solvent (75%) for 24 hours at room temperature [16]. The *Opuntia* seed extract was filtered and dried in a forced-air dryer at 37°C. The residue was redissolved in hydroethanolic solvent and made up to 5 mL [17]. The yield of the extracts was expressed in terms of milligrams of dry hydroethanolic extract per gram of dry plant weight (mg DE/g DPW). The final extract was kept in vials at 4°C until the corresponding analyses were conducted.

2.3. Total polyphenolic content

The total polyphenolic content (TPC) of *Opuntia* seeds extract was determined by the Folin-Ciocalteu reagent method [18]. A reaction mixture of 20 µL of the extract, 1155 µL of distilled water and 100 µL of Folin-Ciocalteu reagent (10%) were prepared. A vigorous stirring was performed and 225 µL of sodium carbonate (10%) was added. After 30 min of incubation at 25°C, the absorbance of the resulting blue-colored solution was measured at 765 nm. A standard curve was prepared by using different concentrations ranging from 0.01 to 0.1 mg/mL of gallic acid. After that, the sample concentration was calculated from the gallic acid standard curve equation ($y=1.11x + 0.099$, $R^2=0.987$) and TPC was expressed as mg gallic acid equivalents per gram of dry extract (mg GAE/g DE). All analyses were performed in triplicate.

2.4. Total flavonoid content

The total flavonoid content was measured spectrophotometrically [19]. This procedure consists of the formation of the aluminum-flavonoid complex. A total of 100 µL of the sample was mixed with 900 µL distilled water and incubated for 5 min. Then, 500 µL $AlCl_3$ (2%) was added to the mixture and incubated for 15 min. Standard calibration curve was prepared with different concentrations ranging from 0.01 to 0.1 mg/mL of quercetin. The absorbance was measured at510 nm and the measurement was compared to a quercetin calibration curve equation ($y=0.1.83x + 0.133$,

https://doi.org/10.56717/jpp.2025.v04i01.032

Table 1. Samples collection sites and their eco-geographic characteristics

Collection sites	Bioclimatic stage	Rainfall (mm/year)	Average Temp. (°C)	Geographical location		
				Longitude (N)	Latitude (E)	Altitude (m)
Kasserine	Arid	254.8	18.4	35°10'20.179"	8°49'50.747"	656
Sidi Bouzid	Upper arid temperate	2304	20.6	34°49'59.99"	9°30'0.00"	327
Gafsa	Lower Arid	185.7	20.8	34°28′1.2′′	9°16′1.2′′	431

Table 2. Total polyphenolic and flavonïd contents of *Opuntia* seeds hydroethanolic extract.

Collection sites	Total polyphenolic content (TPC, mg GAE/g DE)	Total flavonoïds content (TFC, mg QE/g DE
Kasserine	6.92 ± 0.27^{a}	2.85 ± 0.19^{a}
Sidi Bouzid	5.89 ± 0.33^{ab}	2.25 ± 0.38^{a}
Gafsa	5.37 ± 0.41^{b}	2.16 ± 0.56^{a}

Note: values are expressed as means ± Standard Deviations (SD) of triplicate experiments. Means with different letters within the same column are significantly different at 5% using the *Duncun* test (p<0.05).

R^2= 0.998) and the results were expressed as mg of quercetin equivalent per g of dry extract (mg QE/ g DE). All analyses were done in triplicate.

2.5. DPPH radical scavenging activity

The study of the DPPH* free radical scavenging activity of the *Opuntia* seeds extract was performed according to the method described by Brand-Williams [20] with some modifications. Briefly, 500 µL of the sample, at different concentrations, was added to Eppendorf tubes containing 1000 µL of 0.1 mM DPPH*. After 30 min of the reaction at 25 °C in the dark, the scavenging activities of the samples and standards (Ascorbic acid, 1-100 mM) were evaluated by measuring the absorbance at 517 nm. The control consisted of 500 µL of ethanol and 1000 µL of DPPH. All experiments were performed in triplicate. For each sample concentration tested, the inhibition percentage (%I) of DPPH in the steady state was determined following the equation:

$$\% I = [(Abs\ control - Abs\ sample)/Abs\ control] * 100$$

The results were expressed as the inhibitory concentration of the extract needed to decrease DPPH absorbance by 50% (IC_{50}). Concentrations were expressed as mg of ascorbic acid equivalent per gram of dry extract (mg AAE/g DE).

2.6. Statistical analysis

Results are presented as mean ± standard deviation (SD) of three independent experiments. Variance analysis (ANOVA) was performed on measured data. The *Duncan's* test of multiple range was then applied to highlight the significant differences at p<0.05. All experiments were performed in triplicate (n=3).

3. Results and discussion

3.1. Total polyphenolic and flavonoïd contents

The total polyphenolic and flavonoid contents (TPC and TFC) in the *Opuntia* seeds hydroethanolic extract for three regions (Kasserine, Gafsa, and Sidi Bouzid) were evaluated (Table 2). The TPC ranged from 5.37 mg to 6.92 mg gallic acid equivalent/g dry extract (mg GAE/g DE). The TFC varied from 2.16 mg to 2.85 mg quercetin equivalent/g dry extract (mg QE/g DE). The comparison of the total polyphenol and flavonoid contents of the prickly pear seeds ethanolic extracts between the different regions revealed that those of the Kasserine region are the richest polyphenols with 6.92 mg of GAE /g DE and 2.85 mg QE /g DE for TFC and TFC, respectively.

The contents reported by Cardador-Martínez et al. [21], for the same species, in the cultivars of Mexican origin are lower compared to the results obtained in this study and they range from 337 to 460 mg GAE/100 g MS. A study conducted by Nigar et al. [7] reported values higher than our results for local varieties of *ficus ssp*. from Bangladesh. According to Chaalal et al. [22], extracting solvents have a significant effect on the total polyphenolic content. This difference observed in the different studies can be explained by several factors, mainly the low specificity of the Folin-

https://doi.org/10.56717/jpp.2025.v04i01.032

Ciocalteu reagent, the extraction solvent, which carries away non-phenolic substances such as sugars and proteins. Also, the distribution of secondary metabolites such as polyphenols can change depending on climatic conditions, plant maturity, storage conditions, harvest period and geographical location [6, 10, 23, 24]. Furthermore, the extraction method and duration also affect the total content of phenols and flavonoid contents [25]. Several authors have published the important role of polyphenolic compounds on the antioxidant power of the *Opuntia* seeds extract [5, 6, 15, 26, 27]. Polyphenols are mainly accountable for the antioxidant potential of medicinal plants. The total phenolic content can be regarded as an important indicator of the antioxidant properties of plant extracts.

3.2. DPPH radical scavenging activity

The Radical Scavenging Activity (RSA), measured by calculating the ability to scavenge the free radical DPPH, reached the values of 3.55 to 4.97 mg AAE/g DE (Fig. 1).

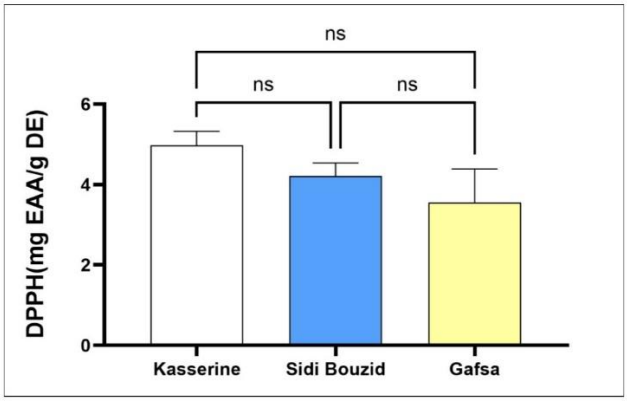

Figure 1. DPPH free radical scavenging activity of *Ounptia* seeds hydroethanolic extract.

All plants have strong antioxidant activity. From these results, it can be deduced that the population of Kasserine is the richest in antioxidants with a content of 4.97 mg AAE /g DE followed by the population of Sidi Bouzid with a value of 4.22 mg AAE /g DE followed by the Gafsa region with a content of 3.55 mg AAE/g DE, which is consistent with previous results concerning polyphenol content. This is directly correlated with their high content of polyphenolic compounds (TPC = 6.92 mg GAE/g DE).

In fact, polyphenolic extracts derived from various plant sources exhibit significant antioxidant properties, playing a crucial role in mitigating oxidative stress-related diseases. Due to their potent bioactive compounds, these extracts are increasingly utilized as natural preservatives and functional ingredients in food products [28].

Therefore, it can be noted that there is a significant positive correlation between the concentration of polyphenols and the antioxidant activity, which confirms that polyphenols are powerful antioxidants capable of inhibiting the formation of free radicals and opposing the oxidation of macromolecules [2, 23, 29]. It can be confirmed that the concentrations of polyphenolic compounds have a significant role in the antioxidative power of the plant extract.

4. Conclusions

This study has investigated the variation in total polyphenolic content (TPC), total flavonoïds content (TFC) and antioxidant activity of hydroethanolic extract of *Opuntia ficus-indica* L. collected from three regions (Kasserine, SidiBouzid and Gafsa) of Tunisia. All plants were found to be rich in polyphenolic compounds and have a good potential antioxidant. These results proved that the plants with high levels of total polyphenolic content are characterized by high antioxidant capacity. *Opuntia* seeds have proven to be an effective potential source of polyphenols and could be useful in replacing or even decreasing synthetic antioxidants in foods, cosmetics and pharmaceutical products. However, more research is warranted to fully elucidate its potential benefits. Moreover, although preliminary evidence suggests that *Opuntia* seeds polyphenolic compounds may possess promising beneficial effects, further in *vitro* and in *vivo* investigations (antidiabetic, antibacterial, and antibiofilm activities) are needed to fully understand its biological properties and potential therapeutic applications in humans.

List of abbreviations

TPC: Total polyphenolic content
TFC: Total flavonoid content
AAE: Ascorbic acid equivalent
DE: Dry extract
OFIK23: *Opuntia ficus* indica kasserine 2023
OFIS-B23: *Opuntia ficus* indica Sidi Bouzid 2023

https://doi.org/10.56717/jpp.2025.v04i01.032

OFIG23: *Opuntia ficus* indica Gafsa 2023
DPPH: 2,2-Diphenyl-1-picrylhydrazyl

Authors' contributions

Conceptualization, K. H.; Methodology, K.H.; H. S., A.A., F.Z., I.T., M. B-Z. and S.K; Formal analyses, K.H., H.S. A.A., F.Z., and I.T.; Investigation, K.H., H.S. and S.K.; Resources, K.H.; Writing-original draft preparation, K.H. and H.S.; Writing-review and editing, Supervision, S.S-E.

Acknowledgements

This work was supported by the Tunisian Ministry of Higher Education and Scientific Research and the Faculty of Sciences of Gafsa.

Funding

This research received no external funding

Availability of data and materials

All data will be made available on request according to the journal policy

Conflicts of interest

The authors declare no conflict of interest.

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