TST ISSN:10020861

ISSN: 1002-0861
Volume 57, Issue 05, May, 2024

Quantitative Analysis and Antioxidant Effects of SEED Extracts of prickly pear (*Opuntia-ficus-indica* L.) from Souk-Ahras, (North-East Algeria)

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Keywords:
Opuntia Ficus Indica; seeds; phenolic contents; antioxidant power

ABSTRACT

A quantitative investigation was undertaken to explore the diverse applications of Opuntia Ficus-Indica. The study, initiated with a biological examination of seeds, focused on specimens gathered in October 2021 from the Sidi-Fredj area in the Souk-Ahras province of North-East Algeria. Four types of organic extracts: Ethanol, methanol, ethyl acetate, and hexane, underwent quantitative analysis, assessing tannins, flavonoids, and total polyphenols content. Results indicated that the methanolic extract exhibited the highest values for all three components (316; 182.51; and 454.33 µg/mL respectively), followed by ethyl acetate extract (283.38; 118.33 and 409.4µg/mL respectively). Total antioxidant activity, as assessed in vitro, revealed moderate in ethyl acetate and methanol, compared to hexane and ethanol (3.91; 3.56; 3.27 and 3.01µg/mL respectively). Methanol extract exhibited the most potent FRAP iron reduction with a CI50 of 22.09µg/mL, while ascorbic acid showed a CI50 of 1.21µg/mL as a positive control. In the DPPH radical reduction test, ethanol extract recorded a CI50 of 2.19µg/mL, followed by ethyl acetate with an IC50 of 1.93µg/mL. In the beta-carotene bleaching test, significant anti-radical activity was observed in methanol, followed by ethyl acetate (AA = 2.04; 1.82µg/mL respectively). These findings provide valuable insights for future biological investigations concerning this plant commonly known as 'el hindi'. Extract exhibited the highest phenolic contents, followed by the ethyl acetate extract .Total antioxidant activity was moderate in ethyl acetate and methanol compared to hexane and ethanol. The methanol extract showed the most potent FRAP iron reduction. In the DPPH test, the ethanol extract recorded a CI50 of 2.19 µg/mL, followed by ethyl acetate with an IC50 of 1.93 µg/mL. Significant anti-radical activity was observed in methanol, followed by ethyl acetate, in the beta-carotene

bleaching test.

1. Introduction

The utilization of natural extracts derived from seeds and plants found in their native habitats has garnered increasing attention due to the myriad benefits associated with their consumption. This has led to a surge in research efforts dedicated to exploring the potential advantages of natural extraction and its applications. As a result, there has been a concerted focus on investigating extracts from various plant species, including those from the Opuntia genus [1]. *Opuntia ficus-indica* (OFI) stands out as a significant source of fruit and is commonly found in semiarid and arid regions across several countries worldwide. Additionally, it is cultivated on suitable terrain to meet industrial demands [2]. *Opuntia ficus-indica*, a resilient xerophytic plant native to Mexico, particularly thrives in arid and semi-arid regions, reaching heights of up to five meters [3]. Introduced to North Africa around the 16th century, *Opuntia ficus-indica* found a hospitable environment in Algeria, characterized by its predominantly semi-arid climate covering 80% of the country's total area. Notably, Algeria boasts approximately 52,000 hectares of *Opuntia ficus-indica*, locally known as 'el hindi,' demonstrating the plant's significant presence in the region [4]. Opuntia, capable of thriving in water-deficient conditions, encompasses a diverse range of species, estimated to be between 200 to 300 [5]. Its resilience in harsh environments stems from its succulent leaves, which serve pivotal roles in thermal regulation, drought resistance, and water retention. Notably, Opuntia holds appeal as a food source due to its efficiency in converting into dry matter, thus providing edible energy. Particularly in forest-adjacent regions, *Opuntia ficus-indica* (OFI) crops serve as vital food resources, especially during periods of drought, supporting both human and local mammal populations. These crops boast high nutritional value, rich in antioxidants such as ascorbic acid and flavonoids, which confer significant health benefits [6].

Since ancient times, humans have relied on medicinal plants for both sustenance and remedies, owing to their abundance of natural bioactive compounds capable of alleviating various toxicities induced by oxidative stress [7].

The *Opuntia ficus indica* plant holds significant economic importance across various sectors, including agriculture, food, pharmaceuticals, cosmetics, and herbal therapy, particularly in the management of conditions such as diabetes and high cholesterol [8- 10]. Cactaceae, the family to which Opuntia belongs, are renowned for their abundance of both primary and secondary metabolites, bioactive compounds, and polyphenols, which play crucial roles in human health by bolstering defenses against pathogens [11]. Consequently, the *Opuntia ficus-indica* plant has become a focal point for researchers worldwide due to the plethora of beneficial components present in its various parts, including fruits, seeds, and cladodes. Notably, the seeds are rich in oil, water, minerals, proteins, and polysaccharides, making them particularly valuable in terms of their chemical composition [12].

Polyphenolic compounds are by-products of plant metabolism. The growing interest in polyphenolic compounds is due to their antioxidant ability [13]. Polyphonic compounds are present in all components of the cactus plant, such as fruit seeds which have high amount of polyphenolic compounds [14].

The literature on cactus plants extensively covers their chemical composition and the presence of antioxidants in various parts, including the seeds [15- 19]. However, despite this wealth of information, seeds have been relatively understudied compared to other parts of the plant. Recent research has increasingly focused on uncovering the natural antioxidants present in seeds. Studies have revealed that seeds from Opuntia species are rich sources of polyphenols, flavonoids, and tannins, with concentrations of these molecules often surpassing those found in the fruit pulp [20]. Given this context, the primary objectives of our study were twofold: to determine the levels of tannins, total polyphenols, and flavonoids in seeds obtained from *Opuntia ficus-indica* using various solvents for extraction; and to assess the antioxidant capacity of these seed extracts. To our knowledge, there have been few reported studies on the chemical composition of seed extracts from *Opuntia dillenii* and their antioxidant activity. This study aims to fill this gap in the literature and provide valuable insights into the antioxidant potential of Opuntia seeds.

2. Material and methods

2.1 Presentation of the study area

The study was carried out using seeds of *Opuntia ficus-indica* sourced from Sidi Fredj, located approximately 34km from the province of Souk-Ahras, Algeria Figure 1. The collection site had an altitude of 450m and coordinates of latitude (N) 35°58'06.9" and longitude (E) 008°15'06.6". The fruits were collected in 2022 and underwent a process of air drying followed by shade drying to obtain hard seeds. Subsequently, the dried seeds were ground into a fine powder using a grocery machine. The resulting powder was then stored under study conditions for subsequent analysis and dosages.

2.2 Preparations of extracts

A total of 75g of finely powdered of Opuntia seeds

Was dissolved in 250mL of various organic solvents, namely methanol, ethanol, hexane, and ethyl acetate.

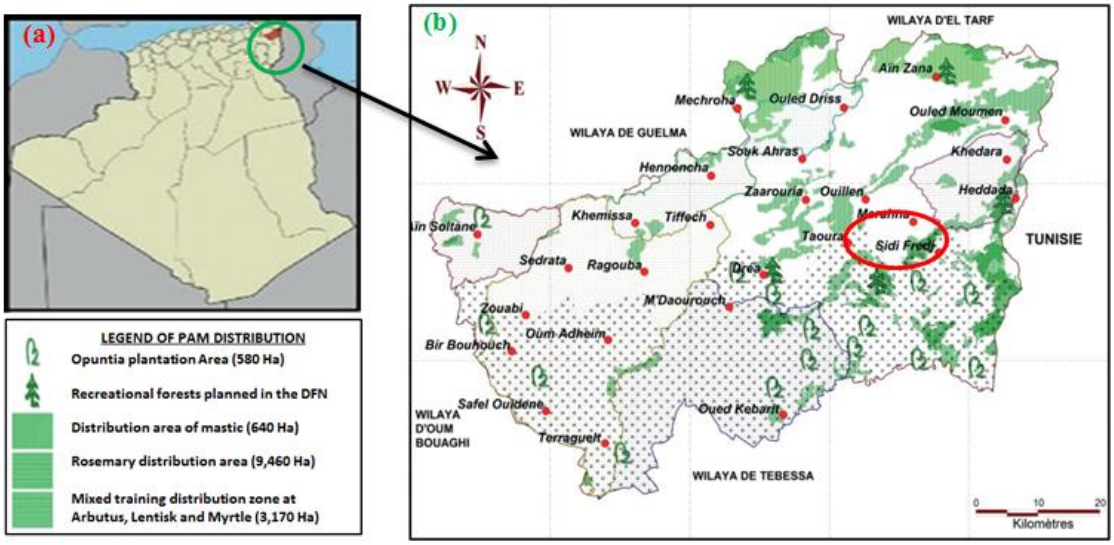

Figure 1: Geographical location of the study area Sidi-Fredj (wilaya of Souk-Ahras)
Source: Souk-Ahras Forest Conservation

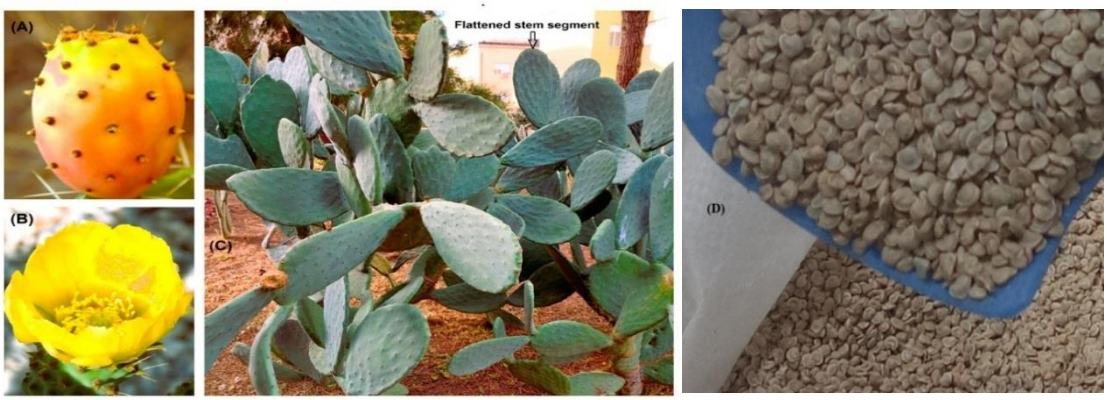

Figure 2: Representation of (A) fruit, (B) flower, and (C) plant and (D) seeds

The mixture underwent vigorous stirring for 15 minutes to ensure thorough mixing. Subsequently, the solution was incubated for 48 hours in a light-proof environment to create an optimal reactive medium. Following incubation, the extract was filtered using Whatman paper to remove any solid particles, and then evaporated to dryness at 45°C using a Buchi steam rotary evaporator. The resulting dry residue was recovered by adding 3mL of the respective maceration solvent for further analysis.

Phenolic compound quantification

Total polyphenolic contents

The study methodology, as presented by [21]. relies on Folin-Ciocalteu reagent as the primary reactive agent. To initiate the assay, 0.5mL of the extract or standard, suitably diluted, is combined with 1.5mL of Folin-Ciocalteu reagent (previously diluted tenfold). Following a 5-minute incubation period, 1.5 milliliters of sodium carbonate solution (6%) are added to the mixture. After incubating the resulting mixture for 1 hour, the absorbance of the solution is measured at 760nm. The polyphenol content of the Opuntia extracts is then determined by comparison with a calibration curve prepared using Gallic acid under similar conditions as the extract.

Total Flavonoid contents

The quantification of flavonoids from seed extracts of *Opuntia ficus-indica* was conducted using the method described by [22]. with some modifications. A reactive medium was prepared by combining 2.5mL of *Opuntia ficus-indica* extract with AlCl3 solution (2% in methanol). The mixture was thoroughly stirred and then incubated for 30 minutes. Subsequently, the absorbance of the solution was measured at 430nm. The flavonoid content was determined by comparison with a calibration curve prepared using Quercetin under the same conditions as the extract.

Total Tanins contents

The quantification of flavonoids from seed extracts of *Opuntia ficus-indica* was conducted using the method described by [22]. with some modifications. A reactive medium was prepared by combining 2.5mL of *Opuntia ficus-indica* extract with AlCl3 solution (2% in methanol). The mixture was thoroughly stirred and then incubated for 30 minutes. Subsequently, the absorbance of the solution was measured at 430nm. The flavonoid content was determined by comparison with a calibration curve prepared using Quercetin under the same conditions as the extract.

Biological activity

Total Anti-radical activity

The anti-radical activity in vitro of fig seed extracts has been evaluated to determine the concentration of fig tree seed, as it is based on the reduction of ions Mo 6+ and Mo5+ is the formation of a phosphate complex, followed by [24]. with quantitative modifications. One 100µl of Opuntia ficus indica extracts was taken, mixed with 1mL of a solution with acid PH forming the sulfuric acid complex (h2so4,0.6M) and sodium phosphate (Na2H2Po4, H2o, 0.6M), then ammonium haptamolybdate (Nh4,6Mo7O24,4h2o,4Mm). the incubation is done for 90min at 95°C, after cooling to ambient temperature. The absorption is read at 695nm, and the total activity was expressed on mg of Gallic acid equivalent per g of dry matter prepared to the same condition of the extracts.

Ferric Reducing Antioxidant Power FRAP

The method of reducing ferric iron from barbaric fig seeds into iron salt in the presence of an agent (chromogen, potassium ferricyanide k3f2 (CN)6 on acidic medium containing trichloroacetic acid [25]. For each fig tree extract 1mL was added to the phosphate buffer and K3fe (Cn6), the mixture is incubated at 50°C for 20 min. 2.5 mL of trichloroacetic acid is added to the reaction medium which is centrifuged for 30min [3000rmp]. Then the overflowing agent is added to the distilled water and the Fecl3.The reading is done at an absorbance of 700nm, the values of IC50% are defined as the concentration of the extract or control with the absorbence of 0.5 [26].

2,2-diphényl 1-picrylhydrazyle assay DPPH

The method described by [27]. Utilizes DPPH as a stable free radical that changes from purple to yellow upon oxidation by antioxidants present in the extracts. In this procedure, 100ul of each *Opuntia ficus-indica* seed extract at various concentrations is introduced into a solution containing 1900ul of methanol and DPPH. The reactive medium is then incubated in a light-protected environment for 30 minutes. Subsequently, the absorbance of the solution is measured at 517nm. The results are expressed as a percentage of inhibition using the following formula: % inhibition = {[Abs cont (-) – Abs samp] / [Abs control (-)]} X100. Here, Abs cont (-) represents the absorbance of the control (containing only the solvent and DPPH), Abs samp is the absorbance of the sample and Abs control (-) is the absorbance of the control sample containing only methanol and DPPH.

The inhibition percentage results obtained for each extract are then compared to a standard antioxidant, such as ascorbic acid, used as a positive control (+), as well as a negative control (-) consisting of 100ul of methanol and 1900ul of DPPH

β-carotene bleaching assay

The beta-carotene bleaching test, as evaluated by [28]. Involves several steps. Initially, 2mg of beta-carotene is dissolved in 10mL of chloroform, to which 2mg of linoleic acid and 200mg of Tween 40 are added. The mixture is then evaporated to remove the polar solvent (chloroform) at 40°C in a 100mL water container saturated with oxygen, while vigorously stirring the mixture. Subsequently, samples of 2.5mL are added to tubes containing 350ul of extracts at different concentrations, followed by a 48-hour incubation period. After incubation, the absorbance of the samples is measured at 490nm. In parallel, a standard antioxidant, such as BHT, is prepared under the same conditions. The anti-radical activity by this test can be determined using the following formula: % inhibition= (1−(Abs contro/Abs sample)) ×100.

Here, Abs_sample represents the absorbance of the sample after incubation, and Abs_control represents the absorbance of the control sample (without extract) after incubation. The percentage of inhibition reflects the anti-radical activity of the extracts.

2. Statistical analysis

The values are represented as the mean ± standard deviation (SD), with n = 3 replicates for each experiment. Statistical analysis is performed using one-way analysis of variance (ANOVA) followed by Dunnett's or Tukey's test for multiple comparisons. A significance level of $p \leq 0.05$ is considered statistically significant. This approach allows for robust comparison between different experimental conditions and determination of any significant differences in the results.

3. Results and discussions

3.1 Phenolic compounds quantification

The results obtained from the barbaric fig seed extracts indicate that the highest phenol content is observed in the methanol extract, with a value of 454.33±2.081µg/mg, as detailed in Table1. This is followed by the acetate, ethanol, and hexane extracts, respectively, based on input to the Gallic acid calibration curve and expressed in µg/mg of extract. Statistical analysis reveals a highly significant difference ($p=0.0001 < 0.001$) in the content of condensed tannins among the studied extract types, leading to the rejection of the null hypothesis (H0). Polyphenols, recognized as secondary metabolites, are prevalent in various parts of medicinal plants [29]. and play a crucial role in antioxidant potential due to their ability to form stable radicals through hydrogen bonding [30], [31].

The tannin content in the studied fig seed extracts varies significantly, with the highest content observed in the methanol extract at 316±14.42µg CE/mg, as indicated in Table 1, This is followed by the acetate, ethanol, and hexane extracts, with tannin contents of 283.38±1.197µg, 192.08±2.07µg, and 94.14 CE/mg, respectively, making hexane the least active extract. The selection of these types of extracts is based on their frequent use in the determination of phenolic compound content, including condensed tannins, which are polyphenols abundant in seeds [32]. These compounds are known for their antioxidant properties across several plants, along with other constituents such as Quercetin, coumarins, and ferulic acids [33], [34].

Table 1. Tanins, flavonoids and phenolic contents in *Opuntia-Ficus indica* seeds extracts

Extracts	Total phenolic (µg/mL)	Tanins (µg/mL)	Flavonoïds (µg/mL)
Methanol	454.33±2.081***	316±14.42***	182.51±2.186***
Ethanol	368,37±0.548	192.08±2.07	46.96±1.002
Ethyl acetat	409.40±0,5254	283.38±1.197	118.33±1.527
Hexane	277.66±2.0816	94.14± 00	79.380±0.541

Data are presented as means ± standard deviation of three repetitions; Analysis of variance (ANOVA) revealed significant effect if ($p< 0.05$).

The statistical analysis reveals a highly significant difference ($p=0.0001 < 0.001$) in the content of condensed tannins among the studied extract types, leading to the rejection of the null hypothesis (H0). This indicates that the content of tannins in seed extracts is significantly influenced by their chemical nature and the extraction solvents used, irrespective of the study conditions. In the context of plants, environmental stressors can trigger the production of phenolic substances, including tannins, as a response to biotic factors such as nutrient deficiencies, drought, and changes in light intensity [35]. This suggests that variations in tannin content observed across different extract types may be attributed to differences in environmental conditions, plant physiology, and extraction methods.

The method described by [22]. reveals that the content of flavonoids is highest in Methanol at 182.51±2.186µg QE/mg, followed by ethyl acetate at 118.33±1.527µg QE/mg. Subsequently, the content

gradually decreases in hexane and ethanol extracts, with values of 79.380±0.541µg QE/mg and 46.96±1.002µg QE/mg, respectively, as depicted in Table 1, Statistically, the difference between the four extracts is highly significant ($p=0.0001<0.001$). Factors such as the high solubility of flavonoids in the study solvents, their chemical nature, the extraction method, and the choice of calibration range may contribute to the variation in flavonoid content among the extracts of medicinal plants. Additionally, the geographical region and extreme environmental conditions of the study area could also play a role in influencing flavonoid levels in plant extracts.

The Barbary fig tree is indeed rich in polyphenols, which have been shown to help reduce high levels of blood glucose and minimize cholesterol levels. Additionally, polyphenols possess natural healing properties against various health issues, including stomach disorders, heart spasms, and headaches. The phenolic content in the extracts, varies among the different extracts, with generally high phenol content observed across most extractions. This observation is consistent with previous research conducted by [14], [36], [37]. Furthermore, the presence of quercetin in the studied extracts serves as a chemical marker, confirming the taxonomical identity of the extracts. This finding aligns with studies conducted by [38]. Overall, these results underscore the potential health benefits of extracts derived from the Barbary fig tree, particularly due to their high polyphenol content and the presence of quercetin.

Compared to another study, our results confirm that obtained by [31]. or barbaric fig seeds are rich on total polyphenols, flavonoids, and tannins (38.99mg EAQ/Ml/ 31.58mgEQ/ml/2.8mgEC/ml respectively). [21]. In the barbaric fig tree analysis, the research results range between 1.55 and 2.64 mg/QE/100 g of DW for flavonoids, and 4.1 to 6.6 mg/CE/100g of Dry weight for tannins.

In this account [39]. State that in the seeds of *Opuntia-ficus-indica* with different extracts, they are rich in phenolic components: the total polyphenols, flavonoids and tannins (from 0.96 to 1.11 mg EQG/g; 0.658mgEQC/g, 0.07 mg EQG /g respectively) [36]. Found in an Algerian species, 144.50mg GAE/100g of DW on total polyphenols, and 44 mg QE/100 g DW for flavonoids.

3.2 Antioxidant activities

The study of total antioxidant activity reveals that ethyl acetate exhibits the highest antioxidant potential, with a value of 3.91±0.74µg/mL, followed by the methanol fraction, hexane, and ethanol, respectively (3.56±1.15µg/mL; 3.27±0.11µg/mL; 3.01±1.02g/mL). This trend is supported by the statistical analysis, which indicates a significant difference between the four extracts ($p<0.05$). The variability in antioxidant activity values observed in Table 2. Can be attributed to several factors, including the chemical composition and richness of polyphenols and flavonoids in the extracts, as well as the nature of the solvent used for fractionation and the presence of other bioactive substances [11]. These findings highlight the importance of considering various factors when evaluating the antioxidant potential of plant extracts and underscore the potential benefits of ethyl acetate and methanol fractions in terms of antioxidant activity.

Table 2. Antioxidant activity of *Opuntia-Ficus-Indica* seed extracts

Extraits	**Total activity µg/mL**	**Reducing Power A0,5µg/mL**	**DPPH test IC50% µg/mL**	**Beta-carotene bleaching AA%**
Methanol	3.56±1.15a	22.09±2.73a	0.89±0,12	2.04±0.46a
Ethyl acetate	3.91±0.74	5.14±0.07	1.93±0.54b	1.82±0.33b
Ethanol	3.01±1.02	6.98±0.55	2.19±1.16a	1.63±0.44
Hexane	3.27±0.11	13.21±0.17b	1.63±0.67	0.51±0.36

Ascorbic acid	---	1.21±0.36	3.31±0,25	---
BHT	---	---	---	46.93±6.93

Statistical analysis is presented as means ± standard deviation of three repetitions; Analysis of variance (ANOVA) revealed significant effect ($p< 0.05$); Different superscript letters represent significant variations.

The reduction capacity test, aimed at reducing ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}), yielded notable results. Methanol exhibited the highest reduction capacity with a strength of 22.09±2.73 µg/mL, followed by hexane with a strength of 13.21±0.17 µg/mL. Ethanol and acetate, however, demonstrated lower reduction strengths at 6.98±0.55 µg/mL and 5.14±0.07 µg/mL, respectively Table 2

These findings suggest that the extracts are rich in phenols and phenolic compounds, particularly flavonoids, which are known for their ability to chelate transitional metals responsible for the formation of hydroxyl radicals resulting from the chemical reaction of iron with hydrogen peroxide [40]. The observed differences in reduction power among the extracts serve as indicators of their antioxidant potential [41], [42]. Statistical analysis using ANOVA revealed significant differences between the tested extracts, with a p-value of $0.0001 < 0.05$, underscoring the significant variance in their reduction capacities. These results further emphasize the importance of considering various antioxidant assays to comprehensively evaluate the antioxidant potential of plant extracts.

The anti-radical activity of the studied extracts varies depending on the method used, as noted by [43]. In this study, the anti-radical potential in seed extract of prickly pear was quantified using ascorbic acid as a standard. The results presented in Table 2, indicate a variation in antioxidant activity between the extracts, with ethanol exhibiting the highest activity with an IC50 of 2.19±1.16µg/mL, followed by acetate, ethanol, and methanol with IC50 values of 1.93±0.54µg/mL, 1.63±0.67µg/mL, and 0.89±0.12µg/mL, respectively. The latter extracts demonstrate lower activity compared to the positive control ascorbic acid, with an IC50 of 3.31±0.25µg/mL. Statistical analysis using ANOVA confirms significant differences between the different extracts ($p=0.015<0.05$), indicating an effect of the extracts on the IC50%. The anti-radical activity of the tested extracts varies depending on the content of phenolic compounds, which are more strongly detected in polar solvents. Phenols are effective hydrogen donors to the DPPH radical due to their chemical structure, as noted by [44]. In the beta-carotene bleaching method, the results presented in Table 2 show a high percentage of anti-radical activity in the methanol extract at 2.04±0.46µg/mL, followed by acetate, ethanol, and hexane with lower antioxidant activity (1.82±0.33µg/mL; 1.63±0.44µg/mL; 1.53±0.36 µg/mL, respectively). The positive BHT control exhibits significantly higher activity compared to the study extracts, with a value of 46.93±6.93µg/mL. ANOVA analysis also indicates significant differences between the extracts in this test ($p<0.05$). The oxidation of linoleic acid generates peroxide radicals, which oxidize slightly unsaturated beta-carotene, causing its discoloration. This process occurs due to abstractions of hydrogen atoms from the diallyl methylene groups of linoleic acid.

By [30], declared for antioxidant activity (for DPPH test the IC50 of 0.04mg/ml for polyphenol extract; 0.06 to 0.18 mg/ ml for flavonoid extract, 0.01 mg/mL for Tanins. The IC50 for the beta-carotene test is between 0.81mg/ml, 2.85mg/mL, and 0.46 mg/ml for phenol extracts, respectively.

The percentages of inhibition of the DPPH radical from our Opuntia ficus-indica seed extracts are almost the same as those obtained by [11]. or it shows an IC50: 0.13; 0.15, and 0.2 mg/mL, which are consistent with those obtaining by Chougui and his collaborators in 2013.

4. Conclusions

The in vitro analyses of barbaric fig tree seeds revealed that the extracts obtained through seed powder maceration are rich in natural bioactive substances. *Opuntia-ficus-indica* is recognized as a valuable medicinal plant globally, with applications across various industries. Our study initially demonstrated that seed extracts exhibit high levels of polyphenols, which contribute to their antioxidant potential. Similarly, significant levels of tannins and flavonoids were observed, with methanol and ethyl acetate consistently yielding the richest extracts in these compounds. The results of biological activity assays, including DPPH, FRAP, and beta-carotene tests, indicated that Opuntia extracts possess considerable antioxidant power across all four extracts studied. In conclusion, our research study presents valuable insights for future researchers aiming to further explore the chemical composition and potential uses of *Opuntia-ficus-indica* seeds. The findings highlight the promising antioxidant properties of these seeds and underscore their potential for further valorization and utilization in various aliment industries.

5. Conflicts of interest

There was no conflict of interest of the authors.

6. Acknowledgments

The authors are grateful to Prof. Dr.Barbaros Balabanlı (Ankara Gazi University, Department of Biology, Developmental Biology and Biotechnology) for the plant identification and to the financial support of the Ministère de l'Enseignement Supérieur et de la Recherche Scientifique (MESRES, Algeria) and NOPALTEC Algeria Cooperative Sidi-Fredj Souk Ahras regions Algeria

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