Eurasian J. Sci. Eng. Tech., 2025; 6(1): 047-052

Eurasian Journal of Science Engineering and Technology

Research article

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Characterization of physiochemical properties of Cactus Cladodes (*Opuntia ficus indica*) grown in Antalya

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Abstract

In the current study, the main purpose is to determine the chemical and physical properties of cactus cladode grown in the rural region of Antalya, the Mediterranean region of Türkiye, since it reveals high-yield leaves for potential consumption. Physical and chemical properties were examined. The moisture content and dry matter did not show extra ordinary results, but its ash content emphasized it as a good source of minerals. Total sugar content (TSC) percent was higher than 5%. The functional bioactive components were determined as 8.85 mg/g dry matter, 1.97 mg/g dry matter, 2.43 mg/g dry matter, and 0.468 mg/g dry matter for the total phenolic content (TPC), the total flavonoid content (TFC), the total flavanol content as well as the ascorbic acid content (AAC), respectively. The results indicated that some of the features were superior compared to literature reports, but some were not. In any case, cactus cladodes from the Antalya region of Türkiye reveal high potential as an alternative source of food for human diet. Its carbohydrate content serves as an energy supply, whereas promising bioactive contents including TPC, TFC, total flavanol content and AAC present high potential as a functional nutrient for human consumption. High antioxidant capacity values also support those findings.

Keywords: Antioxidant capacity, Ascorbic acid, Flavonoid content, Phenolic content, Regional effect

1 Introduction

Starvation is the main problem which the humanity has to confront and needs to find solutions. One of the ways is to look for alternative nutritional sources. Towards this purpose, different types of sources have been released from animal and plant worlds. Those findings are promising sources, but increasing population of humanity leads to continue looking for more sources. Thus, more efforts in this way are still well-come.

Opuntia widely grows plant throughout the world, especially on north hemisphere, although it is an endemic plant type of the American continent [1]. It has started spreading from the American continent to the rest of the world, after Christophe Colomb discovered that continent and now it is possible to see the different species of *Opuntia* everywhere. The spreading of this plant in this wide regional areas all over the world is related to its adaptation ability to extremely ranged climatic and/or soil conditions. Thus, this makes it available for human diet and it goes back to ancient times. For example, before the discovery of the American continent by European explorers, continent's hosts had been harvesting *Opuntia* species more than 9000 years [2]. In other words, *Opuntia* species are important nutritional source of human diet in different forms. However, at this point, it is worth to emphasize that it is not common as a diet for most of the cultures except for the American continent, although it is easy to find and harvest. But this does not mean that it is never consumed out of the American lands. This is not to be avoidable for humanity for a long time, only means to need an extra time to re-discover it for human diet.

As mentioned above, beside of the American continent, it grows in the wide land of world from Mediterranean to Middle & South Africa, and Middle East countries, even in India as well as in Australia [3]. Additionally, *Opuntia* species are cultivated in those regions, especially on the American lands. The total area where *Opuntia* species grow wildly or are cultivated is more than three million hectares [4].

One of the most harvested and consumed species is *Opuntia ficus indica*. It is cultivated in 26 countries [4], since it is the most common species of *Opuntia* which is traded in the world [2]. Additionally, *Opuntia ficus indica* is the well-known species and the products originating from *O. ficus indica* are spread all over the world [3]. Although different parts of this plant species are consumed, the most common part for consumption is its leaves known as Cactus Cladode. Cladode yield from *Opuntia ficus indica* has been reported to vary in the range of 30 – 80 tones/hectare depending on the climatic and soil conditions and region and species [5]. However, new taste and/or source always creates a doubt for consumers before consuming new food for the first time, since it may cause serious problems especially due to directly taken into the human body from the intestinal system. Thus, researchers need to investigate any source for human consumption from all aspects. In this context, nutritional and functional potential as well as physical properties are examined and the findings are shared from different channels

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Recieved: 20.01.2025 Accepted: 23.01.2025 Published: 30.01.2025
doi: 10.55696/ejset.1623788

to inform consumers and to make them feel comfortable towards consumption.

In the current study, the main purpose is to discover the chemical and physical properties of Cactus cladode grown in the rural region of Antalya, Mediterranean region of Türkiye, since it reveals high-yield leaves for potential consumption. According to our best knowledge, there are scare number of studies about Cactus cladode grown around Antalya city [6-8] and this study serves to fill that gap in the literature.

2 Material and methods

2.1 Material

Cactus cladodes *Opuntia ficus indica* were harvested in the region be rural area of Yeşilkaraman Village, Aksu, Antalya, Mediterranean Region, Turkiye. Cladode selection were performed according to maturity and shape levels and they were considered to have uniform in those criteria. Until analysis, cladodes were stored at -18°C.

2.2 Analysis

2.2.1 Physical analyses

2.2.1.1 Moisture content

Cactus cladode was subjected to measurement of moisture content. Sample moisture content was measured gravimetrically by using moisture determination equipment (Kern 085, DBS 60-3, Germany) [9].

2.2.1.2 Color measurement

The surface color of the cladode sample was measured using a colorimeter (High-Quality Colorimeter, 3nh, China) and color parameters of CIE were given as L*, a*, and b* [10]. Chroma (C*) and hue values were calculated from the measured color parameters according to the below equations (Equation (1) and (2)).

$$C^*=(a^{*2}+b^{*2})^{1/2} \quad (1)$$

$$Hue°=\tan^{-1}(b^*a^*) \quad (2)$$

2.2.1.3 Texture analysis

Cactus cladode was sliced, before textural analysis. Slice thickness was 5 mm. As textural properties, skin strength and elasticity values of cactus cladode were measured using a texture equipment (Stable Micro Systems, England). Puncture test was performed and the text parameters were adjusted as 1 mm/sec of speed using a 2 mm P/2 prob.

2.2.1.4 Ash content

Ash content of cladode sample was determined by the method by Williams [11]. Amount of the sample was weighted into a dried/pre-weighed porcelain crucible. Burning of sample was carried in an ash furnace at 550±50°C for 6-7 hours until all organic materials was burned off. Ash content was expressed as a percentage of difference in weight before and after analysis.

2.2.2 Chemical analyses

2.2.2.1 Extraction of bioactives

Extraction procedure reported by Ammar et al. [12] was carried to extract bioactive constituents from cactus cladode. Before extraction, sample was sliced and 2 g of sample was transferred into a glass jar and 20 mL of methanol was added as an extraction solution. The mixture was homogenized for 1 min and transferred into an extraction cell. Extra 10 mL of methanol was used to wash out the remaining particles from the glass jar to the extraction cell. Extraction was carried out on a magnetic stirrer for 2 hours. Liquid phase was separated by filtering the extraction medium through filter paper and filtrate was also centrifuged at 4000 rpm for 10 min at 4°C.

2.2.2.2 Determination of total phenolic content

Total phenolic content (TPC) of cladode sample was determined by Folin–Ciocalteu method [13]. In order to determine TPC, the extract obtained in the previous extraction step was used and the absorbance measurement was performed at 765 nm using a spectrophotometer (T70+UV/VIS spectrophotometer, PC Ins., England). Standard curve was prepared using gallic acid standard and the result was given as mg GAE/ 100g dry matter.

2.2.2.3 Determination of flavonoid content

Methanolic extract was evaporated and dissolved by ethanol before flavonoid analysis. Total flavonoid content (TFC) was determined according to the method by Zaporozhets et al. [11]. Rutin was used as a standard material. TFC of the sample was spectrophotometrically measured at 510 nm and given as mg RE/g dry matter [14].

2.2.2.4 Determination of flavanol content

Similar to TFC, ethanolic extract was used to determine total flavanol content of the cladode sample by reading absorbance at 440 nm (T70+UV/VIS spectrophotometer, PC Ins., England) [15]. The standard curve was prepared using rutin as a standard material and the result was given as mg RE/ g dry matter.

2.2.2.5 Phenolic profile analysis

The phenolic profile of the cladode sample was determined chromatographically using HPLC (LC-20AD pump, SPDM20A DAD detector, SIL-20A HT autosampler, CTO-10ASVP column oven, DGU-20A5R degasser, and CMB-20A communication module), (HPLC-DAD, Shimadzu Corp., Kyoto, Japan). The procedure followed was reported by Karadag et al. [16]. A gram of sliced cladode sample was extracted in a 16 mL of methanol for 2 hours and the extract was filtered through a syringe filter (0.45µm) into an amber vial. Each phenolic was given as mg/g dry matter. The mobile phase was 0.1% of Acetic acid (v/v), 0.1% Acetonitrile (v/v) and gradient flow program was applied (Table 1). Inertrsil® ODS C-18 (250×4.6 mm, ID: 5 µm) was used and the column oven temperature was 40°C. The flow rate was 1 mL/min, duration was 63 min, and injection volume was 1 mL. The detection of phenolics was monitored at 278, 320, and 360 nm.

Table 1. Gradient program of phenolic profile analysis

Time (min)	Mobil Phase A-Acetonitrile (%)
0	10%
2	10%
27	30%
50	90%
60	100%
63	10%

2.2.2.6 Ascorbic acid analysis

The ascorbic acid content (AAC) of cladode sample was determined by HPLC [17]. Three gram of sample was mixed with metaphosphoric acid (4.5%) at volume ratio of 1:12.5, and mixture was homogenized. The extraction medium was centrifuged at 4000 rpm at 4°C for 20 min. The supernatant was filtered through a syringe filter of 0.45µm and 20µL of filtrate was injected to HPLC (Agilent 1260 Infinity, Agilent, USA). Agilent XBD C18 (250*4.6 mm, ID: 5 µm) was used as a column and detection was done at 254 nm at column oven temperature of 30°C. Flow rate was 0.8 mL/min. The total flow duration was 35 min. The flow program was isocratic. L-ascorbic acid was used as a standard. The ascorbic acid content of the sample was given as mg/kg dry matter.

2.2.2.7 Antioxidant content

Two antioxidant activity measurement methods were carried out to determine the antioxidant potential of cladode sample.

α, α-diphenyl-β-picrylhydrazyl (DPPH•) Assay:

Free radical scavenging activity (DPPH) of the cladode sample was determined according to the method by Dorman et al. [18]. A 50 µL of methanolic extract was mixed with 450 µL of reactant (Tris-HCl, 50mM, pH 7.4). One mL of 0.10 mM DPPH• (α, α-diphenyl-β-picrylhydrazyl) was added to the solution and the mixture was incubated at room temperature under dark. Absorbance of sample was read at 515 nm using a spectrophotometer (T70 + UV / VIS spectrophotometer, PG Instruments, England). % inhibition of sample was determined using Trolox standard curve and the result was given as µmol TEAC/g dry matter.

2,2'-azinobis (3-ethylbenzothiazoline-6-sulphonic acid (ABTS•+) Assay:

Antioxidant potential of cladode by ABTS assay was determined following the method by Re et al. [19]. In the analysis 7 mM ABTS•+ solution including 2.45 mM potassium persulphate was prepared and the solution was kept for 12-16 hours to form ABTS•+ radicals. Reading was performed at 734 nm using a spectrophotometer (T70 + UV / VIS spectrophotometer, PG Instruments, England). Initial and 6 min later absorbance values of sample was read and the percent reduction was calculated. The result was given as µmol TEAC (Trolox® equivalent antioxidant capacity)/ g dry matter.

2.2.2.8 Determination of total sugar content

Total sugar content (TSC) of cladode was determined by Lane-Eynon method [20]. The result was given as g/100g dry matter.

3 Results and discussions

Current study investigated cactus cladode (*Opuntia ficus indica*) as a potential nutritional source for human diet. For this purpose, first of all physical and chemical properties of cladode sample is required to be defined. Thus, those relevant parameters were analyzed and the cladode sample results were measured. Analyzed properties were mainly classified into two groups. Table 2 summarized the group one being the representation of physical properties.

Table 2. Physical properties of Cactus cladode (*Opuntia ficus indica*)

Physical Properties	Measured Value
Moisture content,%	92.25 ± 0.01
Dry matter, %	7.75 ± 0.01
Ash content, mg/g dry matter	213.23±2.50
Ash percent, %	21.91±0.12
L*	42.42±10.16
a*	-2.04±3.61
b*	23.44±4.66
C*	28.17±4.90
Hue	94.59±9.39
Skin strength, g.force	187.17±0.07
Elasticity, mm	2.61±0.01

All the results were the mean of three replicates with standard deviation.

The moisture content of the cladode sample was higher than 90%. In literature cladode moisture content of *Opuntia ficus indica* was reported as being around 90% [21-22]. Our finding was coincidence with those results. As well known that, water is the most significant molecule of biological materials, since it is required to manage biochemical reactions and microbiological activities. Thus, having information about moisture content makes it be evaluated for any purpose like processing, cooking, and/or consumption. Dry matter content of cladode sample was also determined based on moisture content. Thus, it can be evaluated this product as a low dry matter plant material. This was considered as a significant property, since that low level requires specific process conditions (for example: high temperature level, long process time for drying). The results of mineral content of cladode and its percent level (>20%) indicated that this plant material is a good source of minerals, but the current results do not reveal details like which minerals were rich in the sample. Thus, further study in this context is required. Percent ash value have been studied and reported results were in the range of 20-25% [21, 23]. The ash percent of cactus cladode in the current study was also in that range. Another important parameter is the cladode surface color. Color parameters were seen to be suitable for a green leaf plant material. Cladodes have a moderate lightness. However, higher lightness value (>65) was reported [24]. Difference from literature one may be attributed to climatic conditions, soil, and regional differences. Due to its surface observed color, a value is too low, whereas b value is high. Ayadi et al. [24] reported a* and b* values as −8.17±0.74 and 25.15 ± 0.61. These results of color parameters were very close to the results of the current study. Additionally, these color parameters are

coincidence with green leaf vegetables in general manner. As being another important color parameter, it is well-known that the hue value is the better representation of the color parameter being close to real observation. In the current study, hue value was calculated as more than 90. In other words, it was very close to the green representation level of hue value (120). Textural properties are important in terms of processing and consumption. Skin strength and elasticity values were seen to be around 187 g. force and 2.61 mm, respectively. If the skin strength was too high, it would be hard to process, consume etc. Similarly, elasticity was also important, since it is a response of the sample against to outer forces.

Another group of properties is chemical ones which are important to evaluate any diet source from nutritional and functional aspects. Thus, the chemical analysis was carried and the amount of some important constituents were measured. The calculated results were given in Table 3.

Table 3. Chemical properties of Cactus cladode (*Opuntia ficus indica*)

Chemical Properties	Measured Value
TSC, g/100 g dry matter	5.63±0.19
TPC, mg GAE/g dry matter	8.85±1.12
TFC, mg RE/g dry matter	1.97±0.01
Total flavanol content, mg RE/g dry matter	2.43±0.82
AAC, mg/g dry matter	0.468±0.006
DPPH, µmol TEAC/g dry matter	17.42±1.42
ABTA, µmol TEAC/g dry matter	54.26±2.00

TSC: Total sugar content; TPC: Total phenolic content; TFC: Total flavonoid content; AAC: Ascorbic acid content. All the results were the mean of three replicates with standard deviation.

As can be seen from Table 3, TSC of the cactus cladode sample was the first parameter and it was more than 5% of solid matrix. This value was corresponding to the soluble sugar % and it was found to be coincidence with the literature value published by Ayadi et al. [24]. In that study, total soluble sugar percent was given in the range of 2-6%, which changed depending on species.

The functional potential of any nutrient is another topic on which researchers should focus on and perform on a detailed investigation. Because besides of nutritional contribution to the human diet, food sources should provide functionality managed by its constituents. Phenolics is coming first in this extent, especially for plant-based food sources. Therefore, TPC, TFC, total flavanol content, and AAC were examined and the measured results were given in Table 3. Ayadi et al. [24] also analyzed the total phenolic content of cladodes for two different species. One of them was *Opuntia ficus indica*. The TPC was reported to vary in the range of 825.81 - 975.82 mg /100 g dry matter and this result was compatible with our finding which was equivalent to 885 mg/100 g dry matter. However, cladode belonging to *Opuntia ficus indica* was reported to have lower TPC (varied from 168.6 to 185.8 mg/100g dry matter) compared to the current result. The difference may be attributed to the regional difference, since the variety used in the study was grown in Korea [23]. TFC was also under investigation and the calculated result was found to be higher than the published one (0.81-1.29 mg/g dry matter) by Lee at al. [23]. Similar to TPC values, it was found that cactus cladode grown in Antalya region was a flavonoid-rich source compared to that grown in Korea. Although it is not common in the literature, total flavanol content was also determined in the current study and the result indicated that this group is 23% higher than flavonoids (Table 3). Finally, ascorbic acid was considered in this study and AAC of the cladode sample was determined. Ascorbic acid is a significant vitamin and being in the human diet makes people healthier, since it contributed to the body's self-defense system. Generally, it is sensitive to environmental conditions like temperature, oxidation etc. Thus, its good source has been taken consumer and researcher interests. In our study, this vitamin was detected at the level of 0.468 mg/g dry matter. The AAC of cladode has been extensively in the literature, as well. Chiteva and Wairagu [25] have declared the AAC of *Opuntia ficus indica* cladode sample as 5.17 ± 0.06 mg /100 g dry matter. This AAC value was lower than that found in the current study. In another study, literature survey was performed and the published review gave information about AAC of cladode, as well [26]. In that review, the range was declared as 7-22 mg/100g dry matter. Our finding was also higher than that published range. On the other hand, Lee et al. [20] have reported a compatible AAC result (71.2 mg/100 g dry matter) for cactus cladode compared to that value in the current study. Aragona et al. [27] have revealed the AAC of cactus cladode as 29 mg/100 g. This amount of AA was lower than the achieved result in the current study.

The antioxidant activity potential of cactus cladode was evaluated by two assays; DPPH and ABTS+ methods. The results of DPPH and ABTS+ were found to be as 17.42±1.42 µmol TEAC/g dry matter and 54.26±2.00 µmol TEAC/g dry matter, respectively. Bioactive compounds of *Opuntia ficus indica* plant parts including cladode may act as electron donors to convert free radicals to more stable products, and this has shown that the scavenging effect increases with the concentration of polyphenols [28]. Opuntia plant part also includes flavanol glycosides and it has been proven that those bioactives reveal a potential as an additive in food and cosmetic, as well as pharmaceutical industries due to their antioxidant power [29].

Besides of bioactive content of cladode sample, its phenolic profile has been also examined and resulted phenols were summarized in Table 4.

As in the literature some of the phenolics (ferulic and qumaric acid) are common with the current study that revealed phenolics, but some reported ones in this study have not been seen in literature [26]. It is well-known that phenolics are secondary metabolites of plants against attacks coming from outside to protect its self. Thus, the phenolic profile is totally dependent on the environmental conditions, whether the plant material has been under microbial attack or not, etc. Thus, an identical profile definition is not possible for phenolic profile of the plant materials.

Table 4. Dominant phenolic compounds detected in the cactus cladode of *Opuntia ficus indica*

Ellagic acid	Caffeic acid	p-Qumaric acid	Ferulic acid
3.988±0.0034	2.196±0.006	0.265±0.008	1.050±0.070

All the results were the mean of two replicates with standard deviation.

4 Conclusions

The results of the current study help to characterize the physiochemical properties of cactus cladode grown in Antalya, Mediterranean Region of Türkiye. One of the biggest problems that the world faces is the starvation and increasing population of humanity, and limited sources of food have pushed researchers to look for alternatives. The raw material of the current study has been evaluated in this extent, and the results were found to be promising towards this purpose. However, this study is only accepted as a first step since only the characterization of the physiochemical properties of cladodes was considered in it. However, to accept it as an alternative, it should be investigated from all aspects, like processing, storage, transport, and its availability for the human intestine system. Thus, the current study was classified former step of this area and more efforts should be required in near future to make picture more clear for humanity.

Conflict of interest

The authors declare that there is no conflict of interest.

Similarity rate (iThenticate): 20%

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