

ALGERIAN WILD GREEN CAROB (*Ceratonia siliqua* L.): PHYSICOCHEMICAL CHARACTERISTICS AND ANTIOXIDANT POTENCY

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ABSTRACT. The main purpose of this study was to determine the proximate composition and physicochemical properties of Algerian wild green carob collected from three separate locations in Jijel province (northeast of Algeria), as well as to investigate its antioxidant potency. The results showed that the three samples were substantially comparable in terms of proximate compositions and physicochemical attributes. They have an acidic pH range and a very low fat content. In terms of phytochemical properties, Texenna green pods had the highest levels of polyphenols, flavonoids, and flavonols, with concentrations of 87.10 ± 0.07 $\mu\text{g GAE/mg DM}$, 4.90 ± 0.06 $\mu\text{g QE/mg DM}$, and 37.0 ± 0.19 $\mu\text{g QE/mg DM}$, respectively. Furthermore, the total antioxidant capacity of the methanolic extracts was determined using the phosphomolybdate method and found to be 154.62 $\mu\text{g AAE/mg DM}$. These promising findings revealed that unripe *Ceratonia siliqua* L. from the Texenna region is a rich source of natural antioxidants that could be employed more broadly in functional food formulations.

KEY WORDS: *Ceratonia siliqua* L., Unripe pods, Proximate composition, Physicochemical properties, Bioactive compounds, Antioxidant potency

INTRODUCTION

Carob (*Ceratonia siliqua* L.) is a perennial plant in the *Fabaceae* family native to the Mediterranean region [1], which is the principal carob production center with an annual production of more than 135,000 tons [2]. The carob tree, part of the Oleo-Ceratonion, is widely spread across Algeria, covering territories from east to west, at low and medium altitudes as well as in semi-arid and humid bioclimates [3]. It tolerates salt stress and drought [4] and grows in warm and dry environments with poor soils [5].

When compared to other Mediterranean fruits, the carob has a distinct phenological profile since it blooms in late summer to autumn (September to mid-November), and the fruit grows and ripens on the tree for an unusually long time of 10-11 months [6]. The cultivation of carob is currently expanding due to increasing demand for its compositional, functional, nutritional, and industrial values, making it an economically significant crop [7]. The carob bean is composed of 90% pulp and 10% seeds.

Carob is high in a variety of beneficial nutrients, including dietary fibers, carbohydrates, minerals, and polyphenolic compounds [8]. The presence of polyphenols has been linked to the bulk of the biological actions and health advantages of carob [9]. Polyphenols, as natural antioxidants, can benefit human health and aid in the prevention of some chronic diseases. Many

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studies have shown that carobs and their derivatives exhibit antiproliferative, apoptotic, and antidiarrhetic activities, as well as antihyperlipidemic and antidiabetic effects, due to their high antioxidant, polyphenol, and fiber content [10]. As a result of their chemical constituents, taste qualities, and nutritional benefits, they hold potentially substantial value for the food sector. However, the maturation stage and variety of fruits have an impact on these bioactive components [11, 12].

The majority of studies on *Ceratonia siliqua* L. have focused on high-quality compositional determinations, like minerals, organic acids, soluble sugars, phenolic compounds, protein content, and in vitro assays of radical scavenging and antioxidant activities of brown (mature) carob pods [13]. In this context, the primary goal of this research is to investigate the proximate composition, physicochemical properties, and antioxidant capacity of Algerian wild green carob obtained from three areas of Jijel province in northeast Algeria.

EXPERIMENTAL

Chemicals and reagents

Sigma-Aldrich, Germany, supplied Folin-Ciocalteu, gallic acid, quercetin, L-ascorbic acid, and 2,2-diphenyl-1-picrylhydrazyl (DPPH). Honeywell Fluka, Belgium, provided sodium hydroxide, petroleum ether, boric acid, sulfuric acid, sodium carbonate, aluminum chloride, sodium phosphate, ammonium molybdate, and methanol. All of the chemicals utilized were of analytical grade. The studies were also carried out using distilled water.

Instruments and apparatus

The samples were weighed using an electronic balance (ARA520, Ohaus Corp., China). The dried carob kibbles were crushed with a grinder (Retsch, Grindomix GM 200, China) and then sieved through a 450-mm CISA sieve in Barcelona. A magnetic stirrer (04803-02, USA) was used to stir the mixtures, and a vortex mixer (Eins-Sci E-VM-A, South Africa) was used to vortex them. A Hanna Hi 2210 pH meter (Hanna Glass Works, Medfield, MA) was used to measure the pH of each carob powder solution. The crude ash fraction was obtained with the aid of an oven muffle (BR-18HM/XD-18HM, China). The Soxhlet extraction unit (S.S. Udyog, IndiaMART, India) was used to evaluate crude fat content, while the Kjeldahl digesting unit (BSWKJ, IndiaMART, India) was utilized to determine crude protein content. The extraction was carried out using a rotary evaporator (Buchi R-300, Switzerland). A UV-Vis spectrophotometer was used to evaluate polyphenols, flavonoids, flavonol levels, and antioxidant activity (Analytik Jena SPECORD 50 PLUS VS AJ-822-0050P-2-R, New Zealand). The tubes were incubated in a Memmert water bath from Germany to determine total antioxidant capacity.

Plant sampling

Wild green carob pods are harvested in March in their unripe state from three regions of Jijel province (northeast of Algeria) (latitude: 36° 49' 13" north, longitude: 5° 46' 00" east, elevation from sea level: 9 m). In 2017, pods with slightly different morphologies were randomly collected from three locations in Jijel province, Algeria: El Emir Abdelkader, Chekfa, and Texenna (Figure 1). These areas have not been studied before for their wild green carob and are also recognized for the quality of their carob trees, which are traditionally used by the local community for healing and in the preparation of traditional foods. The collected samples were then washed under tap water to remove debris, rinsed with deionized water, patted dry with paper towels, and let to dry naturally in the sun for 24 hours. The dried carob kibbles were crushed using a grinder and passed

through a 450- μ m CISA sieve after the seeds were manually removed. The fine powders that resulted were used in all experiments.



Figure 1. Green carob pods from A) El Emir Abdelkader, B) Chekfa, and C) Texenna.

Morphological characterization of green pods

Each elementary sample weighs 1 kg. Three separate metrics were measured for each pod: its length, width, and thickness. The number of pods in 1 kg of each sample was also counted.

Proximate composition and physicochemical characterization of carob powders

The chemical proximate composition and physicochemical properties of carob powders were investigated. All samples were tested for pH, total titratable acidity (TTA), moisture, dry matter, ash, fat, and protein contents using standard analytical procedures of the Association of Official Agricultural Chemists, AOAC. The analysis was performed in triplicate, and the average values were calculated and expressed as mean \pm SD (Standard Deviation).

The pH evaluation of a solution containing 10% (w/v) of each carob powder was carried out using a Hanna Hi 2210 pH meter (Hanna Glass Works, Medfield, MA) [14]. Total titratable acidity (TTA) was determined as a percentage of acetic acid by titration against 0.1 N NaOH [15]. The moisture content was calculated by subtracting the fresh and dry weights of samples after drying them at 105 ± 1 °C until they reached a constant weight [16]. While the dry matter was determined by dividing the fresh and dry masses of samples after drying them at 105 ± 1 °C until weight stabilization [16]. Organic matter burning at 550 °C for around five hours until the sample was free of carbon particles yielded the crude ash fraction [17]. Crude fat content was measured by the Soxhlet method [18], whereas the Kjeldahl method [19] was used to determine crude protein content.

Quantification of phenolic compounds

Extraction method

10 g of each powder sample was macerated in 100 mL of a methanol/water mixture (80:20 v/v) known to have more polar organic properties, for one night at room temperature with gentle agitation. The resulting extract was filtered twice through sterile Whatman filter paper No. 1. The filtrate was then evaporated using a rotary evaporator at 48 °C to remove the methanol, resulting in a dry crude extract [20].

Total polyphenol content in dry extract

The Folin-Ciocalteu colorimetric method was used to determine the total phenolic content [21]. 1.20 mL of Folin-Ciocalteu reagent was added to 0.30 mL of each extract. After 5 min, 1.5 mL of sodium carbonate solution (7.5%) was thoroughly mixed with the mixture. The obtained solutions

were incubated in the dark at room temperature for 2 hours. The absorbance was then measured at 765 nm using a UV-Vis spectrophotometer. The calibration curve for sample quantification was created using various concentrations of gallic acid standard solution. The results were expressed in micrograms of gallic acid equivalents per milligram of extract dry weight ($\mu\text{g GAE/mg}$).

Total flavonoids content in dry matter

The total flavonoid content was determined using the aluminum chloride colorimetric method described by Woisky and Salatino [22]. A volume of 0.5 mL of 2% AlCl_3 methanolic solution was added to 0.5 mL of each extract. After 1 hour of incubation at room temperature, the absorbance at 420 nm was measured. The flavonoid concentration was determined using a calibration curve established with quercetin as the standard. The results are given in micrograms of quercetin equivalents per milligram of dry extract ($\mu\text{g QE/mg}$).

Total flavonol content in dry extract

The total flavonol content in the carob powder extracts was calculated as described by Kumaran and Karunakaran [23]. A mixture of 2 mL of 2% AlCl_3 methanolic solution and 3 mL (75 g/L) sodium acetate was added to 2 mL of methanolic extract. The absorption at 440 nm was read after 2 hours and 30 min of incubation at 20 °C. The flavonol concentration was calculated using the calibration curve with quercetin as the standard. The findings were expressed in terms of micrograms of quercetin equivalents per milligram of dry matter ($\mu\text{g QE/mg}$).

Assessment of the antioxidant activity of the extracts

The antioxidant effectiveness of carob kibble powder extracts was determined using two different tests: the DPPH radical scavenging assay and the phosphomolybdate method.

Radical scavenging activity by DPPH assay

The DPPH method with ascorbic acid as a standard antioxidant was used to assess the antioxidant activity of each carob kibble powder extract [24]. Briefly, 0.1 mL of each extract was mixed with 3 mL of a 0.004% DPPH methanolic solution. The mixture was vortexed and left at room temperature in the dark for 30 min. The absorbance was then measured at 517 nm with a visible UV-SPECORD 50 spectrophotometer. The free radical scavenging activity (RSA) was calculated as a percentage using this formula: $\text{RSA (\%)} = [(\text{Abs control} - \text{Abs sample}) / \text{Abs control}] \times 100$, where Abs control is the absorbance of the control without sample and Abs sample is the absorbance of the sample (extract).

Total antioxidant capacity by phosphomolybdate assay

The total antioxidant capacity of the extracts was determined by the phosphomolybdate method using ascorbic acid as a standard [25]. A 0.3-mL aliquot of each methanolic extract was combined with 3 mL of the reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The tubes were capped and incubated for 90 min in a 95 °C water bath. After the samples were cooled to room temperature, the absorbance at 695 nm was measured against a blank (methanol plus reagent) using a spectrophotometer. The total antioxidant capacity was expressed as micrograms of ascorbic acid equivalent per milligram of dry extract ($\mu\text{g AAE/mg}$).

Statistical analysis

The data were subjected to one-way analysis of variance and expressed as the mean \pm the standard deviation (SD). All statistical analysis were performed using the Statistical Package for Social Sciences, 23.0 (SPSS for Windows; SPSS Inc., Chicago, IL). Significance of differences was defined at $p < 0.05$.

RESULTS AND DISCUSSION*Morphological characterization of pods*

Carob pods vary in size, shape, quality, color, and seed yield depending on the cultivar. Plant genotype, geographical origin, climate circumstances, and harvesting and storage procedures all contribute to these variances [3, 5].

Pod size, as determined by the average value of its length, resulted in two types of harvested pods: slightly long ($14 < L \leq 15$ cm) and slightly short ($10 \leq L < 14$ cm). This classification was based on the work of Tutin *et al.* [26] and Batlle and Tous [5], who reported that the average pod size could range from 10 to 30 cm. In our samples, the pods of Texenna are slightly long (12.83 ± 1.15 cm), whereas the pods of El Emir Abdelkader and Chekfa are slightly short (9.33 ± 0.36 cm and 7.83 ± 0.87 cm, respectively) (Table 1). Texenna pods are longer than those studied by Boublenza *et al.* [27] for Bejaia pod lengths of 10.30 ± 0.31 cm; however, El Emir Abdelkader and Chekfa pod lengths are shorter.

Carob pod width is an important agronomic indicator. It is unaffected by pod size and can provide information not only on the compressed or expanded state of the pod but also on the volume of seeds and pulp. According to Tous *et al.* [28], high-pulp pods produce low seed output. The pods in the Texenna region are the widest, with an average width of 2.26 ± 0.14 cm. Tutin *et al.* [26] and Batlle and Tous [5] found 1.5 to 2.5 cm and 1.5 to 3.5 cm, respectively, which correspond with our findings. Bejaia carob pods had a value of 1.91 ± 0.04 , according to Boublenza *et al.* [27].

There is a substantial and significant ($p < 0.05$) variation in pod thickness among samples. It distinguishes between compressed and bulky pods. It can reach 1 cm in length, particularly in fleshy pods [5]. This variable separated fleshy and voluminous pods with a thickness greater than 0.61 cm from those that are flattened with a thickness between 0.33 and 0.49 cm. Based on these characteristics, we may define our pods as flattened (Table 1). Texenna carob pods are the thickest of any Algerian location investigated by Gadoum *et al.* [29].

The previously measured variables, length, width, and thickness, all have a significant impact ($p < 0.05$) on the number of pods in 1 kg. Indeed, samples with wider and thicker fruit provide the lowest count value, as in the case of Texenna pods.

Table 1. Morphological characterization of green carob pods.

Characteristics	Sample area		
	El Emir Abdelkader	Chekfa	Texenna
Length (cm)	9.33 ± 0.36^a	7.83 ± 0.87^b	12.83 ± 1.15^c
Width (cm)	1.46 ± 0.12^a	1.13 ± 0.11^b	2.26 ± 0.14^c
Thickness (cm)	0.26 ± 0.21^a	0.23 ± 0.08^b	0.56 ± 0.11^c
Number of pods/1 kg	60 ± 3.0^a	110 ± 2.3^b	50 ± 3.0^c

^{a-c}Values in the same line and labelled with different letter differ significantly ($p < 0.05$).

*Physicochemical characterization of green carob powders**pH and total titratable acidity (TTA)*

According to the results in Table 2, our samples have an acidic pH range which differs insignificantly ($p > 0.05$) from 5.15 ± 0.02 to 5.23 ± 0.02 , which compares favorably to the value reported by Abi Azar [30] for Lebanese green carob, which was 5.

Regarding the acidity, green carob powders from the Chekfa region had the highest value, $9.00 \pm 0.1\%$ (Table 2). Our findings outperform those of Benchikh *et al.* [31], who recorded a TTA of $2.53 \pm 0.05\%$. According to Kader and Barrett [32], this acidity is related to the unripe fruit's organic acid content, and as the fruit ripens, organic acid contents decrease due to their use during respiration or conversion into sugars; additionally, these acids can be converted into a variety of substances, including amino acids. Acidity is determined by the organic acid content, which differs between cultivars [33].

Moisture and dry matter contents

The results demonstrate that the moisture content of the three samples ranges from $78.6 \pm 0.3\%$ to $83.4 \pm 0.2\%$, with a substantial rate reported for the sample from the Chekfa region, followed by Texenna, and finally El Emir Abdelkader (Table 2). Our results are greater than those obtained by Ben Othmen *et al.* [34] for carob pods gathered from various Tunisian localities ($63.31 \pm 1.11\%$ to $76.65 \pm 1.13\%$). The moisture content of the El Emir Abdelkader sample is comparable to the percentage of 78.4% reported by Vekiari *et al.* [35].

Ben Othmen *et al.* [34] stated that the moisture content of the fruit decreases as it ripens. The same authors believe that this decreasing moisture pattern is required to sustain the nutritious integrity of mature fruits during storage. This would also increase the amount of dry matter, explaining the hardness of mature pulps. In the same context as Ben Othmen *et al.* [34], we can speculate that the El Emir Abdelkader provenance has the toughest texture and forecast that it will retain its sensory and nutritional features for a longer period of time. It is worth noting that the dry matter percentages of the three locations' green carob pods are extremely close (Table 2). El Emir Abdelkader and Chekfa samples have the same dry matter content, but Texenna has the highest since it contains more pulp.

Table 2. Proximate composition and physicochemical characteristics of carob powders.

Characteristics	Sample area		
	El Emir Abdelkader	Chekfa	Texenna
pH	5.15 ± 0.02^a	5.22 ± 0.12^a	5.23 ± 0.02^a
TTA (% acetic acid)	8.04 ± 0.6^a	9.00 ± 0.1^b	6.96 ± 0.2^c
Moisture content (%)	78.6 ± 0.3^a	83.4 ± 0.2^b	82.6 ± 0.2^c
Dry matter content (%)	9.77 ± 0.0^a	9.77 ± 0.1^b	9.82 ± 0.3^c
Ash content (%)	9.94 ± 0.41^a	10.0 ± 0.23^b	9.98 ± 0.09^c
Crude fat content (%)	0.29 ± 0.22^a	0.15 ± 0.02^b	0.31 ± 0.02^c
Crude protein content (%)	9.43 ± 0.03^a	9.37 ± 0.23^a	9.43 ± 0.03^a

TTA: Total titratable acidity. ^{a-c}Values in the same line and labelled with different letter differ significantly ($p < 0.05$).

Ash content

The ash contents of the three samples are quite comparable but differ significantly ($p < 0.05$), with the Chekfa sample showing a tiny rise with an average percentage of $10.0 \pm 0.23\%$ (Table 2). Our results are higher than the variation interval (3.30–5.16%) found by Ben Othmen *et al.* [34] for

the six Tunisian regions studied. According to Seraglio *et al.* [36], ash content is vulnerable to climate and environmental fluctuations caused by regional origin variance. This pattern could be explained by the fact that mineral salt transfer in the phloem is mostly determined by the environment in which the carob trees grow and the plant's condition.

Crude fat content

According to the data shown in Table 2, our green carob pods have a very low fat content, ranging from $0.15 \pm 0.02\%$ to $0.31 \pm 0.02\%$.

Avallone *et al.* [33] reported a percentage ranging from 0.4 to 0.8%. Vekiari *et al.* [35] published the first research on the quantification of fatty acids in developing Greek carob pods, demonstrating that the percentage of fatty acids varies significantly during pod growth. In the first stage, the pod was high in linoleic and α -linolenic acids, which then converted to oleic acid, followed by saturated palmitic fatty acids. They also stated that oleic acid levels increased throughout the ripening period, whereas linoleic and α -linolenic acid levels declined.

Crude protein content

Protein content analysis revealed that the El Emir Abdelkader and Texenna samples had the same protein content of $9.43 \pm 0.03\%$, while the Chekfa sample had a value of $9.37 \pm 0.23\%$ (Table 2). These results are higher than those reported by Abi Azar [30], Vekiari *et al.* [35], and Benchikh *et al.* [31], who reported amounts of 1.12%, 8.3%, and 6.12%, respectively.

Many studies have revealed that the amino acid composition of carob fruits varies according to species, geographical origin, ripening stage, and cultivation method [26, 37]. It is well known that protein content decreases during ripening due to greater respiration rates as well as a significant increase in protease activity related to resource depletion [38].

Quantification of bioactive compounds

Texenna green pods possessed the highest quantities of polyphenols, flavonoids, and flavonols, as indicated in Table 3: $87.10 \pm 0.07 \mu\text{g GAE/mg DM}$, $4.90 \pm 0.06 \mu\text{g GAE/mg DM}$, and $37.0 \pm 0.19 \mu\text{g GAE/mg DM}$, respectively. Polyphenols were the most important secondary metabolites and were thought to be an interesting molecules that determined the nutritional and functional qualities of the fruit [39]. Our total polyphenol compound results exceed those of Ben Othmen *et al.* [34], who reported a variation interval of 4.57–13.46 mg GAE/g DM. While Saci *et al.* [11] found a value of $258.55 \pm 2.57 \text{ mg GAE/g DM}$ in Bejaia green carob pods in northeast Algeria. According to Qasem *et al.* [40], immature carob cultivated in Yemen has $127.02 \pm 7.18 \text{ mg GAE/g}$. More recently, Ben Othmen *et al.* [12] measured an amount of $11 \pm 0.1 \text{ g GAE/100 g DM}$ for the ethanolic extract of unripe carob cultivated in Tunisia. The variance in polyphenol content might be attributed to numerous factors such as geographical origin [34], extraction circumstances [12], variety, and ripening stage [31, 39].

When it comes to mature fruits, Ayad *et al.* [41] published a value of $23.375 \pm 0.83 \text{ mg GAE/g DM}$ in ripe carob pods from the Texenna region in Jijel. Many authors [12, 31, 34, 39] found that the total phenolic content of carob decreased gradually during ripening to the lowest levels at the end of maturity. This trend was most likely explained by the activation of polyphenol oxidase (PPO), which leads to phenolic component oxidation [42]. Indeed, phenolic compounds had a mild polar character at the start of maturity, but their condensation during ripening conferred significant polarity [39]. This pattern is further confirmed by their role in enzymatic browning.

In terms of flavonoid content, our findings are considerably lower than those of Qasem *et al.* [40], who found $49.74 \pm 0.88 \text{ mg QE/g}$, but higher than those of Benchikh *et al.* [40], who recorded concentrations of $276.93 \pm 4.55 \text{ mg QE/100 g DM}$, respectively. Ben Othmen *et al.* [34]

recorded a range variation of 275.91 - 562.91 mg RE/100 g DM. During the ripening process, flavonoid concentration follows the same pattern as polyphenol concentration. Similarly, Farag *et al.* [43] found that green pods had the highest concentrations of phenolic chemicals, whereas mature pods had the lowest. According to Kappel *et al.* [44], this behavior was mostly due to enzymatic degradation, secondary phenolic chemical conversion, and transfer throughout the ripening process. Benchikh *et al.* [39], on the other hand, explained the decrease in total flavonoid content by the fact that the photosynthetic process pauses when chlorophylls are fragmented, resulting in a break in flavonoids synthesis and subsequent degradation.

However, to our knowledge, there is no similar data on the determination of flavonol content in green and brown carob pods, although Goulas and Georgiou [45] showed that flavonol aglycones, notably quercetin and myricetin, were the most powerful antioxidants in carob extracts. In our study, *Texenna* pod extract had the highest flavonol content, with 37.0 ± 0.1 μ g QE/mg DM.

In general, bioactive compound concentrations were subject to the influence of several factors. Chigayo *et al.* [20] concluded that the phytochemical screening and solvent extraction analysis provide useful guidance on the phytochemicals present in the extracts as well as suitable extraction solvents. They also noted that serial extractions demonstrated that yields can be significantly boosted by doing short, repeating extractions. In another study, Ayad *et al.* [46] highlighted that the processing methods significantly affect the bioactive compound content and the antioxidant capacity. Plant sources and utilized parts also played a key role in the variation in total phenolic, total flavonoid, and antioxidant activity [47, 48].

Antioxidant activity and total antioxidant capacity of extracts

Because of their important role in disease prevention and health benefits, there is increased interest in the antioxidant effects of phytochemical substances found in plant extracts. There is no established method for assessing the antioxidant activity of plant extracts. Antioxidant potential has been estimated using a variety of approaches [39].

To examine the efficiency of antioxidants in samples, two methods (the DPPH assay and the phosphomolybdate method) were used. The DPPH test provided information about the activities of compounds containing stable free radicals; the DPPH effect was thought to be attributable to their ability to donate hydrogen. The antioxidant activity percentages of the three samples studied exhibited extremely close antioxidant activity with a significant difference ($p < 0.05$), with the El Emir Abdelkader sample being the highest with a percentage of $86.00 \pm 0.02\%$ (Table 3). Our results are significantly greater than those of Benchikh *et al.* [39], who reported a value of 30.37 ± 0.03 g AAE/100 g DM for wild Algerian unripe carob. Ben Othmen *et al.* [34] displayed a range variation of 20.98 ± 0.15 g TE/100 g DM to 32.78 ± 0.78 g TE/100 g DM in Tunisian green carob. Qasem *et al.* [40] found a potent antioxidant activity expressed in IC_{50} in methanol extracts of unripe carob pods generated using a Soxhlet extractor (IC_{50} value of 11.23 ± 0.47 μ g/mL). Whereas, Rtibi *et al.* [49], on the other hand, recorded moderate DPPH scavenging activity in immature carob pods (IC_{50} value of 188.22 ± 2.23 μ g/mL). According to Meziant *et al.* [50], the antioxidant activities of fruits cannot be attributed just to their phenolic contents but also to the combined actions of many chemical components.

In terms of total antioxidant capacity, the methanolic extract from the *Texenna* sample had the highest capacity, with a value of 154.62 ± 0.25 μ g AAE/mg DM, while the other two extracts, from El Emir Abdelkader and Chekfa, had values of 53.46 ± 0.52 μ g AAE/mg DM and 38.03 ± 0.38 μ g AAE/mg DM, respectively. Ayad *et al.* (2022) [41], found a percentage of 48.89 ± 0.24 mg AAE/g DM for mature *Texenna* carob. Total antioxidant capability decreased as compared to the percentage of unripe fruit. The antioxidant capacity changed in the same way as the bioactive compound content, confirming the significant link between bioactive compound levels and antioxidant capacity. Although the effect of ripening on the biochemical composition and

antioxidant capacity of carob pods has been extensively studied [12, 34, 35, 36, 39, 43], the origin impact has been demonstrated since each provenance has its own unique climatic, geographical, and pedological characteristics, such as rainfall levels, sun exposure, and temperature, all of which influence the production of phenolic compounds [34]. The intriguing bioactive component richness and significant antioxidant capacity verified the remarkable nutritional and functional potential of this fruit in its unripe state, particularly its Texenna provenance.

Table 3. Bioactive compound content and antioxidant capacity of green carob powder extracts.

Assays	Sample area		
	El Emir Abdelkader	Chekfa	Texenna
Total polyphenols ($\mu\text{g GAE/mg DM}$)	36.74 \pm 0.01 ^a	45.14 \pm 0.04 ^b	87.10 \pm 0.07 ^c
Total flavonoids ($\mu\text{g QE/mg DM}$)	4.09 \pm 0.11 ^a	3.95 \pm 0.20 ^b	4.90 \pm 0.06 ^c
Total flavonols ($\mu\text{g QE/mg DM}$)	16.22 \pm 0.41 ^a	7.80 \pm 0.15 ^b	37.0 \pm 0.19 ^c
Antioxidant activity (%)	86.00 \pm 0.02 ^a	85.40 \pm 0.12 ^b	85.09 \pm 0.32 ^c
Total antioxidant capacity ($\mu\text{g AAE/mg DM}$)	53.46 \pm 0.52 ^a	38.03 \pm 0.38 ^b	154.62 \pm 0.25 ^c

GAE: Gallic acid equivalent, QE: quercetin equivalent, AAE: ascorbic acid equivalent, DM: dry matter.

^{a-c}Values in the same line and labelled with different letter differ significantly ($p < 0.05$).

CONCLUSION

Ceratonia siliqua L., a member of the *Fabaceae* family, is one of the medicinal plants of great therapeutic significance. The physicochemical properties data revealed that our samples had a high total titratable acidity (TTA), moisture, ash, and protein content but a low crude fat level. These samples are also being investigated for phenolic component content and antioxidant activity in their methanolic extracts. Polyphenols were found to be the most important secondary metabolites present, reflecting the nutritional and functional properties of the fruit. On the other hand, Texenna had the highest amounts of bioactive compounds (polyphenols, flavonoids, and flavonols) among the three tested regions. Unripe carob extracts also demonstrated significant percentages of free radical scavenging activity and a surprising powerful total antioxidant capacity, which was always from the Texenna region. Because of its vital health-promoting characteristics, there is a growing interest in adding carob extracts to food items, either to develop functional foods or to avoid chronic diseases. As a result, utilizing our extracts, particularly those of Texenna, as an excellent source of natural antioxidants for application in the medicinal and food sectors is of interest.

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