

The Influence of Altitude on the Polyphenols Content and Antioxidant Capacity of Northern Moroccan 'Dellahia' Prickly Pear Juice

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Abstract: - Moroccan cactus exhibits high genetic variability with several cultivars. The 'Dellahia' prickly pear variety, prevalent in northern Morocco and noted for its green pulp, is among the least valued cactus varieties, primarily consumed fresh. This study aimed to assess the impact of altitude on total phenolic acids and flavonoid content (TPC and TFC) and the antioxidant activity of 'Dellahia' prickly pear juice from northern Morocco. Significant differences in TPC ranged from 91.29 to 130.45 mg GAE/Kg of juice from the Mestassa and Wahran sites (at 119 m and 482 m altitude, respectively). TFC also varied slightly, from 18.8 to 19.1 mg RE/Kg of juice. Variations in antioxidant activity were evident in both DPPH• and ABTS+ assays, with DPPH• inhibition percentages ranging from 8.85% to 19.14% and ABTS+ inhibition from 41.07% to 54.35%. However, the influence of altitude on these parameters was inconclusive, as samples from higher altitudes did not consistently yield lower or higher values. Other factors such as soil composition, sunlight, and farming practices may influence these results.

Key-Words: - *Opuntia ficus-indica*, Dellahia, altitude, polyphenols, flavonoid, antioxidants, DPPH, ABTS.

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1 Introduction

There are over 1,500 species and 130 genera in the *Cactaceae* family, 300 of which are in the *Opuntia* genus, [1]. The prickly pear cactus, *Opuntia ficus indica*, is found in the Mediterranean region, Central and South America, Central and South Africa, the Middle East, and India, [2], [3], [4]. Cactus has been used fresh or processed for human consumption, as well as a functional constituent for food and pharmaceutical products due to the important content of bioactive compounds like polyphenols, [5], [6], [7], [8]. The edible pulp and pericarp of the fruit might be soft green, greenish-white, canary yellow, lemon yellow, red, cherry red, or purple, [9]. The cactus pear crop has several ecological and economic benefits. Unfortunately, Morocco's output suffers a significant loss due to a lack of monetization opportunities. Cactus pear is also gaining popularity in numerous countries because of its ecological,

environmental, and socioeconomic benefits, including erosion and desertification control, and fruit-producing feed, [10]. Its ecological benefits are due to its crassulacean acid metabolism, which facilitates CO₂ absorption at night, reducing water loss during photosynthesis, [1], [4], [11]. Cactus pear is suited for growth in marginal dry and semi-arid environments because of its low water requirements and high-water use efficiency ratio, [12], [13]. The Food and Agriculture Organization recommends cactus pear as a potential crop in light of global climate change, [12], [14]. According to [15], cactus pear is a low-input crop that can be grown sustainably and yield fruits and cladodes that are edible to both people and animals. There are many different species of Moroccan cacti, and they exhibit a high degree of genetic variation, [10]. They are categorized according to the following: when they flower (early or late), what color the blossoms are

(yellow, orange, or pink), what color the fruit and pulp are (green, yellow, orange, red, or purple), what shape the fruit is (oval, round, or rectangular), what their organoleptic characteristics are [16], and how much antioxidant they contain, [17], [18]. The ‘Dellahia’ prickly pear, which is common in northern Morocco, is distinguished by its green pulp. Because of the poor oil content of its seeds, it is one of the least valuable cactus kinds. As a result, its fruits are mostly consumed raw. The purpose of this study is to discuss the effect of altitude on total polyphenols and flavonoid content, and antioxidant activity in the fruit juice of this variety in northern Morocco to reevaluate the many options for reducing the loss of excess production.

2 Materials and Methods

2.1 Vegetal Materials

Prickly pear fruits were collected in August 2016 from four sites at different altitudes in northern Morocco as shown in Table 1 (Appendix). The global view of the study region and the sampling sites are shown respectively in Figure 1 and Figure 2.



Fig. 1: View of the study region



Fig. 2: Sampling sites

2.2 Chemicals and Reagents

Folin-Ciocalteu reagent (2N), DPPH• (2,2-Diphenyl-1-picrylhydrazyl), ABTS+ (2,2'-Azinobis-3-Ethylbenzothiazoline 6 Sulfonic Acid), rutin, gallic acid and methanol were obtained from Sigma Aldrich Corp. (Merck KGaA, Darmstadt, Germany). Sodium carbonate (Na_2CO_3), sodium nitrite (NaNO_2), sodium hydroxide (NaOH), potassium persulphate ($\text{K}_2\text{S}_2\text{O}_8$), aluminum chloride (AlCl_3) and Trolox (6-hydroxy-2-aluminumtramethylchroman-2-carboxylic acid, purity $\geq 97\%$) were purchased from Fisher Scientific SA. (Boulevard Sebastien Brant - F67403 Illkirch Cedex – France). All of the other reagents were of analytical grade unless otherwise stated.

2.3 Determination of Total Polyphenols and Flavonoid Content

2.3.1 Determination of Total Polyphenols Content (TPC)

The total polyphenols content of samples (TPC) was determined spectrophotometrically using the Folin-Ciocalteu reagent method depicted by [19] with minor modifications. Indeed, this reagent is reduced to tungsten and molybdenum oxide in an alkaline medium giving a blue color in the presence of polyphenols. Briefly, the assay was carried out by adding 1 mL of Folin-Ciocalteu reagent (50%) to 0.2 mL of sample extract into a 10 mL test tube. After shaking well, the mixture, 0.8 mL of 7.5% sodium carbonate solution (Na_2CO_3) was added to the mixture. The final mixture was incubated in the dark at room temperature for 30 minutes. Then, we measured the absorbance at 765 nm with an S-22 UV/VIS spectrophotometer. A calibration curve was established using gallic acid. The gallic assay is prepared using various concentrations of gallic acid solutions (from 0 to 1 $\mu\text{g}/\text{mL}$). The gallic range undergoes the same treatment as the sample to be analyzed. Total polyphenol content is determined graphically using the gallic range calibration curve, which is a straight line with the following equation :

$$Y = aX + b$$

Where :

- Y : measured absorbance
- X : total polyphenol concentration required.
- a and b : constants

Then, we expressed the total polyphenols content (TPC) in mg gallic acid equivalent (mg GAE / Kg of juice).

2.3.2 Determination of Total Flavonoid Content (TFC)

The total flavonoid content (TFC) of samples was determined spectrophotometrically following the method depicted by [19] with some minor adjustments. This method is characterized by the formation of a flavonoid-aluminum complex, whose maximum absorbance is 430 nm. The assay was carried out by adding 1 mL of sample extract to 4 mL of double distilled water into a 10 mL test tube. Then, we added 0.3 mL of 5% NaNO₂ solution. After 5 minutes, we added 0.3 mL of 10% methanolic AlCl₃ solution. At the 6th minute, we added 2 mL of 1M NaOH solution. The mixture was made up to 10 mL with double distilled water, and the final mixture was shaken very well. Then, we immediately measured the absorbance at 510 nm with an S-22 UV/VIS spectrophotometer. The calibration curve was established using rutin. A calibration curve was established using rutin. The rutin assay is prepared using various concentrations of rutin solutions (from 0 to 1 µg/mL). The rutin range undergoes the same treatment as the sample to be analyzed. Total flavonoid content is determined graphically using the rutin range calibration curve, which is a straight line with the following equation :

$$Y = aX + b$$

Where :

- Y : measured absorbance
- X : total flavonoid concentration required.
- a and b : constants

Then, we expressed the total flavonoid content (TFC) as mg rutin equivalent (mg RE/Kg of juice).

2.4 Determination of Antioxidant Activity

2.4.1 Determination of Antioxidant Activity by the DPPH Method

Free radical scavenging activity of the methanolic extracts was determined spectrophotometrically following the DPPH• stable radical method depicted by [20] with some minor adjustments. When reacting with an antioxidant compound, the DPPH• radical is reduced by donating hydrogen. This reduction is featured by color changes, from deep violet to dark yellow. Briefly, we added 3.9 mL of a freshly prepared methanolic DPPH• solution (0.1M) to 0.1 mL of samples or positive control of Trolox. We used an equal amount of methanol (4 mL) as a negative control. We performed all measurements in triplicate. After incubating at room temperature in the dark for 30 min, we measured the absorbance at 515 nm with

an S-22 UV-Vis spectrophotometer. Then, we calculated the DPPH• inhibition percentage as the absorbance decrease of the antioxidant samples relative to the control. We determined the Trolox equivalent antioxidant capacity (TEAC) by using the equation established from linear regression after plotting known solutions of Trolox (20–800 µM). At last, we expressed the antioxidant activity as the DPPH• free radical inhibition percentage according to the following formula:

$$\% \text{ Inhibition (DPPH}\cdot\text{)} = [(OD_{\text{cont}} - OD_{\text{samp}})/OD_{\text{cont}}] \times 100$$

With:

- OD_{cont}: Optical Density (absorbance) of the control (pure methanol)
- OD_{samp}: Optical Density (absorbance) of the sample

The effective concentration required to inhibit 50% of the free radical (IC₅₀) is calculated using the equation of the curve obtained by plotting the different inhibition percentages of free radical DPPH• as a function of different sample concentrations of total polyphenols contents. Generally, the equation of this curve is linear. We expressed the IC₅₀ in mg/mL of juice.

2.4.2 Determination of Antioxidant Capacity by the ABTS Method

The antioxidant capacity was also determined using a second method based on the ABTS+ scavenging potential as depicted by [21] with some minor adjustments. First, we generated the ABTS+ radical by reacting 7 mM ABTS and 2.45 mM potassium persulphate (K₂S₂O₈). After incubation in the dark at room temperature for 16 hours, we diluted the solution with 80% methanol to obtain an absorbance of 0.70 ± 0.02 at 734 nm. Then, we added 3.9 mL of this ABTS+ solution to 0.1 mL of the sample. The mixture was carefully stirred, and incubated at room temperature for 6 min. Then, we immediately measured the absorbance of the reactive mixture at 734 nm with an S-22 UV-Vis spectrophotometer and compared it to the antioxidant power of Trolox used as a reference. We performed all determinations in triplicate. We determined the inhibition percentage of ABTS+• free radical as the decrease in absorbance of the samples over the control. We also determined the Trolox equivalent antioxidant capacity (TEAC) by using the equation established from linear regression after plotting known solutions of Trolox (20–800 µM). At last, we expressed the antioxidant activity as the ABTS+• free radical inhibition percentage according to the following formula:

$$\% \text{Inhibition (ABTS+)} = [(OD_{\text{cont}} - OD_{\text{samp}}) / OD_{\text{cont}}] \times 100$$

With:

- OD_{cont} : Optical Density (absorbance) of the control (pure methanol)
- OD_{samp} : Optical Density absorbance of the sample

The effective concentration required to inhibit 50% of the free radical (IC_{50}) for the ABTS+ assay was determined by the same graphical method described previously for the DPPH• assay.

2.5 Statistical Analysis

We used SPSS 20 software and Microsoft Excel 2019 to perform descriptive statistical analysis (based on the calculation of the mean and standard deviation for each parameter studied), analysis of variance (one-way ANOVA test of variation: "sites"), and comparison of means. We used the Student-Newman-Keuls test to determine significant differences between means with a 95% confidence interval ($P = .05$).

3 Results and Discussions

3.1 Total Polyphenols (TPC) and Total Flavonoid Contents (TFC)

Total polyphenols and flavonoid contents (TPC and TFC) exhibited by the different samples studied are displayed in Table 2 (Appendix). The calibration curves of gallic acid and rutin are displayed in Figure 3 and Figure 4, respectively.

Phenolic compounds, also known as polyphenols, are metabolic products widely distributed in plant foods; they possess many biological and pharmacological properties that may offer protection against chronic diseases. These compounds have an antioxidant effect superior to that of vitamins; they can neutralize the effects of oxidative free radicals [22]. For instance, studies have provided evidence that oral administration of citric acid during insulin-induced hypoglycemia can attenuate increases in oxidative stress biomarkers in brain and liver tissue, reduce increases in serum aminotransferases, and provide histological protection against liver damage [23]. In our study, as displayed in Table 2 (Appendix), we noticed no significant difference between the samples regarding the TPC, with values ranging from 91.29 ± 6.01 to 130.45 ± 12.31 mg GAE/Kg of juice for Mestassa (119 m asl) and Wahran (482 m asl) samples, respectively. This means that the effect of altitude on the total polyphenols content of 'Dellahia' prickly pear juice

from the study area is not significant. The values found in our study are higher than those reported by [24] for the Moroccan prickly pear cultivar Moussa characterized by its yellow pulp (7.76 mg GAE/Kg of juice) and Moroccan cultivar El Asri with red pulp (15.34 mg GAE/Kg of juice). In recent studies, [25] and [26] reported higher values of total phenolic content in prickly pear juice ranging from 310.0 to 511 ± 2.9 mg GAE/Kg of juice and 130 to 180 mg GAE/L of juice respectively.

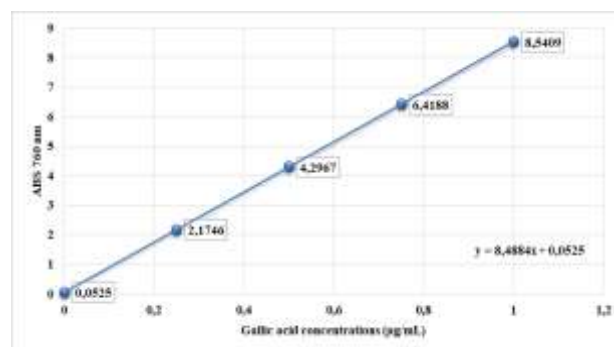


Fig. 3: Gallic acid calibration curve

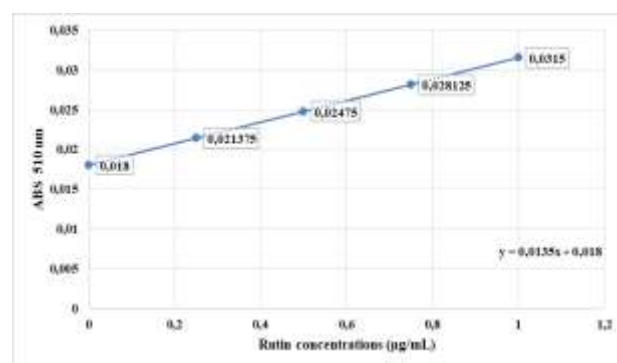


Fig. 4: Rutin calibration curve

3.1.1 Total Polyphenols Content (TPC)

Investigations on red and yellow cultivars from the Kingdom of Saudi Arabia showed that the pulps juices of red cactus contain high total phenolic contents equivalent to 1065.15 mg GAE/100 ml of juice, whereas the juices from the pulps of yellow cactus have lower total phenolic contents (667.82 mg GAE/100 ml of juice), [27]. These values remain higher than those found in our study. In general, our findings are in agreement with the literature considering that previous studies reported a content of phenolic compounds, in *Opuntia* spp. juice, ranging from a minimum of about 22 mg GAE/Kg to a maximum of about 660 mg GAE/Kg, [4], [26], [28], [29], [30], [31]. Fruits such as grapes, apples, pears, cherries, and berries contain up to 200-300 mg of polyphenols per 100 g of fresh weight. Products made from these fruits also contain significant amounts of polyphenols, [32], [33]. The TPC of

'Dellalia' prickly pear juice in the present study was less than that of the other juices such as turnip juice (772 mg/L), red grape juices (1728 mg/L), red wine (1869 mg/L) [34], and pomegranate (1284 to 9476 mg GAE/L of juice). In light of these values, prickly pear and its by-products remain interesting sources of TPC.

3.1.2 Total Flavonoid Content (TFC)

Flavonoids constitute the largest part of polyphenols (2/3), and phenolic acids such as gallic acid and caffeic acid represent the remaining part (1/3), [35]. Flavonoids are classified into several groups, anthocyanins, flavonols, flavones, and flavanones being the most important, [36]. Multiple types of flavonoids have been reported in the *Opuntia* cactus, their kinds and contents differ depending on the variety and degree of ripening, [37]. Today, the properties of flavonoids are widely studied in the medical field for their anti-viral, anti-tumor, anti-inflammatory, anti-allergic, and anti-cancer activities, [38].

In our study, flavonoids constitute less than 1/5 of total polyphenols. As displayed in Table 2 (Appendix), we noticed a significant difference between the samples regarding their total flavonoid content. The samples from Tizakhte (highest altitude, 713 m asl), show the highest total flavonoid content (19.1 ± 0.1 mg RE/Kg of juice) followed by the samples from Mestassa with 18.9 ± 0.2 mg RE/Kg of juice (lowest altitude, 119 m asl). The samples of Wahran and Boujibar situated at respectively 482 and 572 m asl have the same flavonoid content (18.8 ± 0.01 mg RE/Kg of juice). Based on this study, we can establish the effect of altitude on flavonoid content; however, the variability found between samples from different altitudes could also be due to other factors not investigated in our study such as growing conditions for example.

TFC ranging from 50.24 to 52.58 mg RE/Kg respectively for Achefri and Amouslem cultivars at the Arbaa Sahel site in southern Morocco (327 m asl) and 67.02 to 69.95 mg RE/Kg for the same cultivars respectively from Asgherkis site also in south Morocco (709 m asl) have been reported by [10]. These values are higher than those found in our study but confirm that the effect of altitude on the flavonoid content of prickly pear fruits is significant. Investigations on red and yellow cultivars from the Kingdom of Saudi Arabia showed that the pulps juices of red cactus contain high total flavonoid contents equivalent to 159.49 mg RE/100 ml of juice, whereas the juices from the pulps of yellow cactus have lower total phenolic contents (80.35 mg RE/100 ml of juice), [27]. These values remain higher than

those found in our study. In recent studies, higher values of TFC in different cultivars of prickly pear juice have been reported ranging from 47 to 87 mg QE/Kg of juice, [25], [31]

In general, the TFC reported in this study was lower than previously reported values in cactus pear fruits [25], [29], [39], [40], probably because we processed only the pulp without the skin, which should show a higher phenolic content. This explanation is in agreement with data reported in the literature on the polyphenol composition of the skin and pulp of several fruits [17], considering that polyphenols might tend to accumulate in the dermal tissues of the plant body due to their potential role in protection against UV radiation, acting as attractants in fruit dispersal, and as chemical defense against pathogens and predators, [41]. The TFC of 'Dellalia' prickly pear juice in this study was less than that of apple juice (92 mg RE/L of juice) [42], but pretty similar to pomegranate juice TFC (14,45 to 56.99 mg RE/L of juice), [43]. In light of these values, prickly pear and its by-products remain interesting sources of TFC.

3.2 Antioxidant Activity

Antioxidants are compounds that protect cells from the oxidative effects of reactive oxygen species, and an imbalance between these reactive oxygen species and antioxidants results in oxidative stress. Oxidative stress can cause cellular damage associated with a variety of diseases, including diabetes, cancer, cardiovascular disease, neurodegenerative disorders, and aging. Oxidative stress can also damage many biological molecules, with proteins and DNA molecules being important targets of cellular damage. Antioxidants protect cells from damage caused by these free radicals by interfering with the radical-generating systems and enhancing the function of endogenous antioxidants, [44]. Phenolic compounds, and polyphenols in particular, are natural molecules with numerous physiological properties, given their ability to protect cells against damage caused by free radicals, [45], [46]. From a dietary point of view, their intake has a beneficial effect on human health, improving intestinal inflammation, [47], [48] and indirectly interfering with specific signaling proteins, which mediate gene regulation in response to oxidative stress and inflammation, [49], [50].

In general, in this work, the antioxidant activity with ABTS+• was higher than that obtained with DPPH• radical (Table 3, Appendix) with a percentage of inhibition of ABTS+• radical ranging from 41.07 ± 4.47 and 54.35 ± 0.37 respectively for Tizakhte (713 m asl) and Mestassa (119 m asl) samples while the percentage of inhibition of the DPPH- radical ranged

from 8.85 ± 1.13 to 19.14 ± 2.73 respectively for Boujibar (572 m asl) and Tizakhte (713 m asl) samples. These tests showed that the richest sample in total phenolic content was not the one that showed the highest value in free radical scavenging activity against both free radicals ABTS^{•+} and DPPH[•]. The different behavior against both free radicals, which was also observed in other fruits' extracts, could be explained by the fact that many antioxidants react rapidly while other radicals may react slowly, or even be inert, to DPPH[•] due to their steric inaccessibility, [51]. Overall, the results obtained are of the same order as those previously reported [17], [51], [52], but do not confirm that fruits with the highest level of total polyphenols have the greatest free radical scavenging capacity. Indeed, this apparent relationship between total polyphenols and antioxidant capacity was not found in some Mexican *Opuntia* fruits, [28].

In addition, a pairwise correlation between TPC, TFC, antioxidant activity, and altitude was performed to obtain an overall perspective of the free radical scavenging capacity and respective chemical components on the one hand, and the effect of altitude on the different parameters on the other hand (Table 4, Appendix). The Pearson correlation test was used for this purpose. This correlation test showed that the chemical parameter with a significant correlation with the ABTS^{•+} test was total flavonoid ($R=0.885$). In contrast, the ABTS^{•+} and DPPH[•] test values were not significantly correlated with TPC (correlation coefficients of 0.411 and 0.400, respectively). Our results are in contrast to the data reported in the literature by [53] who showed that the correlations between ABTS^{•+} and DPPH[•] tests with TPC and TFC were also high, demonstrating that both tests can be considered to measure the free radical scavenging capacity of these fruits. The same author reported that the chemical parameter with the highest correlation with the ABTS^{•+} test was TPC (0.9818), while the DPPH[•] test had the highest correlation with TFC (0.9735).

The effective concentration to inhibit 50% of the free radical (IC_{50}) was calculated using the equation of the curve obtained by plotting the different percentages of free radical inhibition as a function of TPC sample concentrations. No significant differences were noticed regarding the IC_{50} values for the ABTS^{•+} test. The values found ranged from 90 ± 13 to 120 ± 16 mg GAE/g of juice for Mestassa (119 m asl) and Tizakhte (713 m asl) samples, respectively. As for the IC_{50} of the DPPH[•] test, a significant difference was noticed between the samples, with Tizakhte fruits being more effective (0.222 ± 0.013 mg/mL juice) while Boujibar fruits

being the least effective (0.765 ± 0.034 mg/mL juice). Overall, the IC_{50} values obtained from the ABTS^{•+} assay ranged from 0.090 ± 0.013 to 0.120 ± 0.016 mg/mL of juice for the Mestassa (119 m asl) and Tizakhte (713 m asl) samples, respectively. These values are lower than those obtained by the DPPH[•] assay, demonstrating that all samples are more efficient in free radical scavenging capacity by the ABTS^{•+} assay than by the DPPH[•] assay.

Overall, the correlation between the studied parameters and altitude was not significant according to the Pearson correlation test at 95% and 99% confidence intervals. As shown in Table 4 (Appendix), the correlation coefficients between altitude and the studied parameters ranged from 0.038 and 0.557 for the inhibition percentage of ABTS^{•+} and DPPH[•], respectively. In light of these results, the effect of altitude on TPC and TFC, and antioxidant activity could not be determined.

4 Conclusions

In summary, our study confirmed that prickly pear fruit juice is an important source of polyphenols and flavonoids. Values found are relatively higher or lower than those reported in the literature. A significant difference in total polyphenols, and flavonoid content was observed. Nevertheless, the effect of altitude on these phytochemicals' content could not be established because samples from the highest altitude did not present the lowest or highest content of phytochemicals. For instance, Tizakhte samples located at the highest altitude (713 m) contain 110.96 ± 9.76 mg GAE/Kg of juice while Boujibar, Wharan, and Mestassa samples respectively located at 572m, 482m, and 119 m above sea level contain respectively 109.66 ± 6.79 , 130.45 ± 12.31 , and 91.29 ± 6.02 mg GAE/Kg of juice. As for free radical scavenging activity, the same observation was made using both tests, ABTS^{•+} and DPPH[•]: a significant difference was noticed between samples but this difference is not relevant to the altitude. Indeed, the Pearson correlation test shows that the parameters studied in our assay are not correlated to the altitude. Several factors could probably explain this lack of correlation as growth conditions like the influence of dew on crops from high altitude, that make them grow at similar conditions as if they were at sea level. Other factors as soil composition or sunlight might probably influence these parameters. Further investigations should be carried out with a disposal able to master the influence of external factors.

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APPENDIX

Table 1. Geographic coordinates and climatic characteristics of the study sites

	Altitude (m)	Latitude	Longitude	Mean Temperature (°C)	Annual Rainfall (mm)
Tizakhte	713	35.00597	-4.47130	10 – 30	700 – 800
Boujibar	572	35.01616	-4.46961	10 – 30	600 – 700
Wahran	482	35.03564	-4.46452	10 – 30	500 – 600
Mestassa	119	35.10460	-4.41633	10 – 30	400 – 500

Table 2. Total Polyphenols and Flavonoid contents (TPC and TFC) (Mean ± SD; n = 3)

	Mestassa	Wahran	Boujibar	Tizakhte
Total Phenol Content (TPC)	91.29 ± 6.02 ^a	130.45 ± 12.31 ^b	109.66 ± 6.79 ^c	110.96 ± 9.76 ^c
Total Flavonoid Content (TFC)	18.97 ± 0.2 ^{a,b}	18.81 ± 0.1 ^a	18.80 ± 0.1 ^a	19.11 ± 0.1 ^b

Values in the same line followed by the same letter are not significantly different with a 95% confidence interval.

Table 3. Inhibition percentage and IC₅₀ for DPPH• and ABTS+ assays (Mean ± SD; n = 3)

	Mestassa	Wahran	Boujibar	Tizakhte
% Inhibition DPPH•	10.29 ± 1.37 ^a	16.4 ± 3.52 ^b	8.85 ± 1.13 ^a	19.14 ± 2.73 ^b
% Inhibition ABTS+	54.35 ± 0.37 ^a	54.17 ± 2.42 ^a	45.95 ± 5.70 ^{a,b}	41.07 ± 4.47 ^b
DPPH• IC ₅₀ (mg/mL)	0,326 ± 0,017 ^a	0,634 ± 0,019 ^b	0,765 ± 0,034 ^c	0,222 ± 0,025 ^d
ABTS+ IC ₅₀ (mg/mL)	0,090 ± 0,013 ^a	0,094 ± 0,090 ^a	0,090 ± 0,011 ^a	0,120 ± 0,016 ^a

Values on the same row followed by the same letter are not significantly different with a 95% confidence interval

Table 4. Pearson Correlation matrix for TPC, TFC, free radical scavenging capacity, and altitude

	% Inhibition DPPH•	TPC	% Inhibition ABTS+	TFC	Altitude	IC ₅₀ _ABTS+	IC ₅₀ _DPPH•
% Inhibition DPPH•	1						
TPC	0.400	1					
% Inhibition ABTS+	0.179	0.411	1				
TFC	0.074	0.316	0.885**	1			
Altitude	0.557	0.510	0.038	0.091	1		
IC ₅₀ _ABTS+	0.693*	0.101	0.335	0.405	0.509	1	
IC ₅₀ _DPPH•	0.343	0.406	0.860**	0.776**	0.122	0.509	1

* P-value = .05; statistically significant correlation at the 95% confidence level

** P-value = .01; statistically significant correlation at the 99% confidence level