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Total phenolics, total flavonoids and antioxidant activity of *Moringa oleifera* grown in different locations of Giresun-Türkiye

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Abstract: In this study, nitrogen (N), protein, total phenolics, total flavonoids and antioxidant activity were investigated in dried samples of the *Moringa oleifera* plant grown in Bulancak, Çamoluk, Espiye, Şebinkarahisar and Tirebolu districts of Giresun province in Türkiye. The analyses were performed by DPPH and FRAP methods for antioxidant activity. In the study, the plants grown in Bulancak location had significantly higher nitrogen, protein and antioxidant activity than other growing regions. On the contrary, significantly lower nitrogen and protein were measured in plants grown in Espiye and Tirebolu. The total phenolics and total flavonoids were determined the highest in plants grown in Espiye and Tirebolu locations, the lowest values in Çamoluk and Şebinkarahisar locations. According to the antioxidant activity tests, the antioxidant activity of the plants grown in Espiye location was significantly higher than in other growing regions. The lowest antioxidant activity was measured in Şebinkarahisar and Çamoluk locations. As a result, it was revealed that region conducted the cultivation affects on the protein and bioactive compounds of the *Moringa oleifera* plant. Espiye, Bulancak and Tirebolu locations can be recommended for cultivation.

Keywords: Antioxidant, DPPH, flavonoids, *Moringa oleifera*, phenolics, protein

Giresun-Türkiye'nin farklı lokasyonlarında yetiştirilen *Moringa oleifera*'nın toplam fenolikler, toplam flavonoidler ve antioksidan aktiviteleri

Öz: Bu çalışmada Giresun iline bağlı Bulancak, Çamoluk, Espiye, Şebinkarahisar ve Tirebolu ilçelerinde yetiştirilen *Moringa oleifera* bitkisinin kurutulmuş örneklerinde azot (N), protein, toplam fenol, toplam flavonoid ve antioksidan aktivitesi incelenmiştir. Antioksidan aktivitesi için DPPH ve FRAP testlerine göre analizler yapılmıştır. Araştırmada, Bulancak ilçesinde yetiştiriciliği yapılan bitkilerin, diğer yetiştiricilik bölgelerine göre önemli derecede daha yüksek azot, protein ve antioksidan aktivitesine sahip olduğu tespit edilmiştir. Espiye ve Tirebolu'da yetişen bitkilerde önemli derecede daha düşük azot ve protein ölçülmüştür. Toplam fenol ve toplam flavonoid bakımından, Espiye ve Tirebolu'da yetişen bitkilerde en yüksek; Çamoluk ve Şebinkarahisar'da yetişen bitkilerde en düşük değerler belirlenmiştir. Antioksidan aktivite testlerine göre, Espiye'de yetişen bitkilerin antioksidan aktivitesinin diğer yetiştiricilik bölgelerine kıyasla önemli derecede daha yüksek olduğu saptanmıştır. Aksine en düşük antioksidan aktivitesi, Şebinkarahisar ve Çamoluk lokasyonlarında ölçülmüştür. Sonuç olarak, yetiştiricilik yapılan bölgenin *Moringa oleifera* bitkisinin protein ve biyoaktif bileşikleri üzerine etkisinin olduğu açığa çıkarılmıştır. Yetiştiricilik için Espiye, Bulancak ve Tirebolu lokasyonları tavsiye edilebilir.

Anahtar Kelimeler: Antioksidan, DPPH, fenol, flavonoid, *Moringa oleifera*, protein

1. Introduction

In the rapidly developing world, people's changing living conditions and eating habits and the increasing interest in healthy nutrition constantly increase the interest in products with different tastes, consumption possibilities, and rich nutritional content. One of these products is *Moringa oleifera*. *Moringa oleifera* is the most well-known and cultivated species of the *Moringa* genus in the world. *Moringa oleifera*, whose homeland is the north of India, is grown in countries such as Indonesia, Sri Lanka, Malaysia, Philippines, Mexico, South and Central America, Africa and Middle East. Although it grows well in tropical and subtropical climates, especially in sandy soils, its lack of soil selectivity, formation of pile roots, high adaptability to cold and high drought tolerance has caused *Moringa oleifera* to be cultivated and spread in more expansive areas around the world in recent years (Anwar et al., 2007). The wide spread of the *Moringa oleifera* plant has caused it to gain different local names in many regions. These are brief; Mulangay, Drumstick tree, Mlonge, Kelor, Horseradish tree, Marango, Benzolive, Saijihan and Sajna (Fahey, 2005).

Moringa oleifera is described as a miracle tree, due to its rich content of bioactive compounds, mineral and protein content. Especially the leaves contain high levels of vitamin C, vitamin B_6, provitamin A, vitamin E, beta carotene, protein, potassium, phosphorus, sodium, sulfur, zinc, copper, iron, amino acids, calcium and magnesium. These make a significant contribution

to antioxidant capacity (Bharali et al., 2003; Anwar et al., 2007; Lea, 2010; Moyo et al., 2011). Due to the rich nutritional content of its seeds, roots and leaves, its use as a raw material in the pharmaceutical, food, dye and animal feeding industries has become widespread. Its seeds are used in oil, perfume and hair care, and its wood is used in lumbering. The leaves are used in daily nutrition, salads and animal nutrition. In addition, it is consumed in daily diets because it has a high nutritional content and gives a feeling of satiety when consumed. Also, its leaves are dried and used to increase the shelf life of foods due to its anti-bacterial properties and being a source of disinfectants and natural antioxidants. It is also reported to be used as a dietary product because it gives a feeling of satiety (Nellis, 1996; Siddhuraju & Becker, 2003; Fahey, 2005; Anjorin et al., 2010; Khalafalla et al. 2010; Mishra et al. 2011).

This study aimed to determine the protein, total phenolics, total flavonoids and antioxidant capacity of *Moringa oleifera* grown in different districts (Bulancak, Camoluk, Espiye, Şebinkarahisar and Tirebolu) of Giresun province of Türkiye.

2. Materials and Methods

2.1.Plant materials

Moringa oleifera (cv. PKM-1), which constitutes the plant material of the research, was grown from seed. When the plants reached 10-15 cm in size, they were planted by hand in the first week of June 2019, with 1.0 m row spacing and 0.8 cm row spacing in different districts of the Giresun province of Turkey (Bulancak, Camoluk, Espiye, Şebinkarahisar and Tirebolu). 90 days after planting, which they were about 120 cm high, the stem and branches of the plant were cut by hand from the plant, then stems and leaves were separated with hand, and sun-dried at 30 °C for 3-5 days. Only leaves were used in the analyses. The samples obtained from each cultivation region were ground into powder by milling using a grinder (Waring, UK).

Table 1. Climate data of study areas
Çizelge 1. *Çalışma alanlarının iklim verileri*

Climate data	Bulancak	Çamoluk	Espiye	Şebinkarahisar	Tirebolu
Mean. temperature (°C)	24.9	21.4	24.3	24.8	24.2
Max. temperature (°C)	26.0	26.7	25.4	26.3	26.0
Min. temperature (°C)	18.5	12.4	19.1	12.0	18.7
Rainfall (mm)	13.8	8.3	18.4	3.6	20.6
Relative humidity (%)	73.0	54.0	78.5	55.2	79.0

2.2.Climate date

Climate data were presented in Table 1.

2.3.Nitrogen and protein content

Nitrogen was detected according to the Kjeldahl distillation method (Bremner, 1965). The protein content of *Moringa oleifera* was calculated from the nitrogen content (N=6.25) as determined by the Kjeldahl method.

2.4. Total phenolics, total flavonoids, antioxidant activity

A suspension of 5% *Moringa oleifera* leaves was prepared in pure water and stirred at 45°C for 8 hours. The samples were then filtered on Whatman filter paper 1. The filtrates were used to determine total phenolics, total flavonoids, and antioxidant activity (Ozturk et al., 2019).

Spectrophotometric measurements for total phenolics, total flavonoids and antioxidant activity were performed at the UV-Vis spectrophotometer (Shimadzu, Kyoto, Japan). Total phenolics were determined following the method described by Singleton and Rossi (1965) and were expressed as g GAE (gallic acid equivalent) kg^{-1} drying weight (dw). Total flavonoids were measured according to the method described by Chang et al. (2002) and were expressed as g QE (quercetin equivalent) kg^{-1} dw. The antioxidant activity of *Moringa oleifera* was determined according to two different procedures of 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) (Aglar et al., 2017) and Ferric Ions (Fe^{+3}) Reducing Antioxidant Power (FRAP) (Benzie & Strain, 1996), and the results were expressed in mmol Trolox equivalent (TE) kg^{-1} dw.

2.5. Statistical analysis

Whether the data was typically distributed was checked by Kolmogorov-Smirnov Test. Levene's test confirmed homogeneity control of the group variances. After the variance analysis of the data, Tukey's multiple-comparison test was used to check whether there were significant differences between treatments. The statistical analyses were performed by using SAS software (SAS 9.1 version, USA).

3. Results and Discussion

3.1. Nitrogen and protein

The nitrogen content was between 1.53% and 2.80%; the protein content varied between 9.54% and 17.50%. The nitrogen and protein contents of samples among locations were significant differences. The nitrogen and protein content of samples in Bulancak location were

significantly higher than other locations. Again, significantly higher nitrogen and protein were determined in plants grown in Şebinkarahisar compared to Espiye and Tirebolu. Whereas, similar levels of nitrogen and protein were measured from Çamoluk, Espiye and Tirebolu locations. The protein and nitrogen levels were changed from 9.54% to 16.04% and from 1.53% to 2.57%, respectively. Also, Tirebolu locations samples for nitrogen (1.53%) and protein (9.54%) were the lowest among all locations (Table 2).

Proteins are organic compounds formed by the combination of many amino acids and provide the necessary energy for the body to repair, strengthen and regenerate tissues damaged in human health, regulate metabolism, strengthen the immune system (Çetiner & Bilek, 2018). Therefore, consumers' interest in foods with high protein content is increasing day by day. In our study, it was observed that the nitrogen and, accordingly, the protein content varied according to the locations.

Table 2. Protein and nitrogen content of *Moringa oleifera* grown in different locations
Çizelge 2. *Farklı lokasyonlarda yetiştirilen Moringa oleifera'nın protein ve azot içeriği*

Locations	N (%)	Protein (%)
Bulancak	2.80 a	17.50 a
Çamoluk	2.57 bc	16.04 bc
Espiye	2.51 c	15.67 c
Şebinkarahisar	2.67 b	16.69 b
Tirebolu	1.53 c	9.54 c

Means in columns with the same letter do not differ according to Tukey's test at P<0.05.

It was determined that the plants samples Bulancak location had a higher content compared to other growing regions. As a matter of fact, it has been reported in studies that genetic and climatic factors (temperature, lighting, etc.) and cultural practices such as irrigation and fertilization may affect protein content (Makinde, 2013; Sarwar et al., 2018; Sarwar et al., 2020). According to Gidamis et al. (2003) the protein content of *Moringa oleifera* leaves grown in Tanzania was 33.12%; Moyo et al. (2011) 30.30% in South Africa; Nouala (2006) West Africa 23.27%; Sanchez et al. (2006) found it to be 26.7% in Nicaragua. It has been observed that the reported values are higher than our findings. This difference is because the climates where the studies are carried out are within the natural cultivation areas of *Moringa oleifera*; The reason may be that our locations have a lower temperature compared to the climate in which the research was conducted.

3.2. Total phenolics, total flavonoids, antioxidant activity

In terms of bioactive compounds, significant differences were seen between locations. Total phenolics were between 68.23 and 92.30 g GAE kg^{-1} dw; total flavonoids content ranged from 37.75 to 70.29 g QE kg^{-1} dw. Similar levels of total phenolics and total flavonoids were measured in Espiye and Tirebolu locations, significantly higher than in other locations. However, samples of Çamoluk and Şebinkarahisar locations had similar levels of lower total phenolics and total flavonoids than samples of Espiye, Tirebolu and Bulancak locations (Table 3).

Moringa oleifera contains high amounts of polyphenols, flavonoids, ascorbic acid and phenolic acids. Due to its rich nutritional content, it has been used as a therapeutic in traditional medicine from past to present (Ma et al., 2020). However, it has been reported that this rich content may differ according to geographical location and environmental conditions (Panwar & Mathur, 2020). Indeed, in a study conducted by Saini et al. (2014), *Moringa oleifera* plants grown in Pakistan contain higher bioactive compounds than those grown in India, Thailand, Nicaragua, and the USA. The total phenolic content of *Moringa oleifera* leaves grown in Mexico was between 241.3 and 468.4 µg GAE/mL; it has been reported that the total flavonoids content is between 107.9 and 316.3 µL RE/mL (Coz-Bolanos et al., 2018).

Table 3. Total phenolics, total flavonoids and antioxidant activities of *Moringa oleifera* grown in different locations
Çizelge 3. *Farklı lokasyonlarda yetiştirilen Moringa oleifera'nın toplam fenolikler, toplam flavonoidler ve antioksidan aktiviteleri*

Locations	Phytochemical characteristics			
	Total phenolics (g GAE kg^{-1} dw)	Total flavonoids (g QE kg^{-1} dw)	FRAP (mmol TE kg^{-1} dw)	DPPH (mmol TE kg^{-1} dw)
Bulancak	77.78 b	63.22 b	40.83 b	21.60 a
Çamoluk	71.51 c	41.68 c	31.15 c	13.62 c
Espiye	92.30 a	70.29 a	47.40 a	23.78 a
Şebinkarahisar	68.23 c	37.75 c	26.41 d	12.20 c
Tirebolu	89.32 a	67.35 a	37.52 b	16.78 b

Means in columns with the same letter do not differ according to Tukey's test at P<0.05.

Gómez-Martínez et al. (2020) in 3 different regions in Spain, and Siddhuraju and Becker (2003) in their research in Nicaragua determined that the regions where cultivation is carried out have a significant effect on antioxidant activity. Similarly, in our study, differences in total phenolics, total flavonoids and antioxidant activity were detected between growing regions. It can be stated that this difference is caused by climatic differences such as light, temperature and humidity in the cultivation region. As a matter of fact, in our research, it was observed that low altitude growing regions (locations of Espiye, Bulancak and Tirebolu) had higher bioactive compounds compared to high altitude regions such as Çamoluk and Şebinkarahisar locations.

4. Conclusion

As a result, it has been determined that the *Moringa oleifera* plants grown in different districts of the Giresun province of Türkiye have differences in protein, total phenolics, total flavonoids and antioxidant activity, and plants grown in the Black Sea coastal climate with relatively subtropical climate conditions at low altitude contain higher bioactive compounds. Espiye, Bulancak and Tirebolu locations can be recommended for cultivation. There is a need for more detailed studies on adaptation, yield and nutrient content in different climate conditions of Türkiye.

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