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Original Article / Araştırma Makalesi

INVESTIGATION OF MELATONIN CONTENT AND ANTIOXIDANT CAPACITY IN GRAPE BERRIES

Üzümsü Meyvelerde Melatonin İçeriği ve Antioksidan Kapasitenin İncelenmesi

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ABSTRACT

In this study, six berry fruits; goji berry/wolfberry (*Lycium barbarum*), black mulberry (*Morus nigra*), white mulberry (*Morus alba*), blackberry (*Rubus fructicous* L.), purple mulberry (*Morus rubra*) and Bursa Black mulberry (unknown) were analyzed by HPLC-FD to determine whether they present melatonin. The melatonin was detected different amounts in all samples. Besides, the total phenolic content and antioxidant capacity were studied in all fruit samples. DPPH and ABTS tests were applied to determine the antioxidant capacity. It was observed that the amount of melatonin in the fruit samples varied between 123.44 and 1600.48 ng/g_{FW}. It was found that the concentration of melatonin in goji berry (BF1) was higher than other berry fruits. The highest total phenolic content and antioxidant capacity to were obtained from purple mulberry (BF6) as 276.83 mg GAE/100 g_{FW}, 452.38 mg TE/100 g_{FW} (DPPH) and 555.73 mg TE/100 g_{FW} (ABTS), respectively. Results reveals a significant positive correlation between the total phenolic content with antioxidant capacity.

Keywords: Antioxidant capacity, Berry fruits, Melatonin, Total phenolic content.

ÖZ

Bu çalışmada üzümsü meyvelerden; kurt üzümü (*Lycium barbarum*), horum dutu (*Morus nigra*), beyaz dut (*Morus alba*), böğürtlen (*Rubus fructicous* L.), mor dut (*Morus rubra*) ve Bursa siyah dutunda melatonin varlığı durumu HPLC-FD sistemi kullanılarak belirlendi. Tüm örneklerde farklı miktarlarda melatonin tespit edildi. Ayrıca meyve örneklerinde toplam fenolik içerik ve antioksidan kapasite çalışıldı. Antioksidan kapasiteyi belirlemek için DPPH ve ABTS testleri uygulandı. Meyve örneklerindeki melatonin miktarının 123.44 ile1600.48 ng/g_{FW} arasında değiştiği görüldü. Kurt üzümünde (BF1) bulunan melatonin derişiminin diğer üzümsü meyvelere göre daha yüksek olduğu bulundu. En yüksek toplam fenolik içerik ve antioksidan kapasite mor duttan (BF6) sırasıyla 276.83 mg GAE/100 g_{FW}, 452.38 mg TE/100 g_{FW} (DPPH) ve 555.73 mg TE/100 g_{FW} (ABTS) olarak elde edildi. Sonuçlar, toplam fenolik içerik ile antioksidan kapasite arasında pozitif yönde anlamlı bir korelasyon olduğunu göstermektedir.

Anahtar kelimeler: Antioksidan kapasite, Melatonin, Toplam fenolik içerik, Üzümsü meyveler.

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INTRODUCTION

In recent years, fruits have become increasingly popular as an important source of antioxidants. Thousands of biochemical compounds with different properties, quantities, and functions have been identified in some fruit species including berry species (Ergun & Zarifikhosroshahi, 2020; Gündeşli, 2020). Berry fruits attract attention due to their beneficial effects on human health. In parallel with this increasing interest, the cultivation of such fruits is also becoming widespread. It is possible to come across berry fruit species that grow naturally in almost every region of Turkey. As well as these fruit species are collected from forest areas and sold in local markets, they also available orchards established for commercial reasons. The production of berry fruits in Turkey is 8% on the world scale (Engin & Boz, 2019).

Among the berry fruit species, there are many fruits such as mulberry, blackberry, strawberry, rosehip, kiwi, goji berry and blueberry (Akbulut, Yazıcı, Bakoğlu, & Göksu, 2017). These fruits can be effective in preventing many diseases due to the phytochemicals they contain. Free radicals produced in the human body from metabolic events or other reasons have a major effect on disease occurrences such as cardiovascular, cancer and other diseases. Although the harmful effects of these radicals are eliminated by the defense mechanisms in the body, the body should be supplemented with foods containing natural antioxidants by diet (Ekbul, 2004). The most important of these foods are vegetables and fruits. Berry fruits, which are rich in vitamin C and secondary metabolites such as phenolic acid, anthocyanin, flavonoid and carotenoid, come to the fore with their high antioxidant capacity (Shui & Leong, 2006; Wang, Cao, & Prior, 1996). Berries with different colors are widely used as a natural food coloring and nutritional supplements, thanks to their high antioxidant capacity. As it used in the pharmaceutical industry, it is utilized in the food industry making dried fruit, jam, marmalade, fruit juice, tea, ice cream and cake, too (Engin & Boz, 2019).

Melatonin (N-acetyl-5-methoxytryptamine) is an indoleamine structurally related to other substantial substances such as tryptophan, serotonin, and indole-3-acetic acid. In most living things, melatonin is a biological regulator of various processes such as mood, sleep, and sexual behavior. It has been suggested that this component discovered in plants in 1995 has a physiological role in processes such as flowering, photoperiodicity, and growth (Arnao & Ruiz, 2007). In studies investigating the biological effects of melatonin, its therapeutic effects and health benefits have been extensively studied. It has been emphasized that it has many bioactivities such as antioxidant activity, anti-inflammatory effect, boosting immunity, anticancer activity, cardiovascular protection, anti-diabetic effect, neuroprotective and antiaging activity. Melatonin has been determined qualitatively and quantitatively in animal foods and edible plants in recent years. It has been stated that the consumption of foods containing melatonin increases the serum melatonin level and antioxidant capacity in humans (Anisimov et al., 2006; Chen et al., 2016; Li et al., 2013; Meng et al., 2017; Oxenkrug, Requintina, & Bachurin, 2001; Pandi Perumal et al., 2013). For this reason, the foods containing melatonin have become popular.

In the literature, plant organs such as mostly the flower, leaf, seed, shoot, and root were examined in studies researching the melatonin content in horticultural plants (Arnao, 2014; Chen et al., 2003; Meng et al., 2017; Yan, Shi, & Gong, 2020; Zohar, Izhaki, Koplovich, & Ben-Shlomo, 2011). Especially in the berry fruit group, the studies on the melatonin content of the fruit are limited. The present study proposed to research the melatonin and total phenolic content and antioxidant capacities of goji berry, blackberry, and four different mulberry varieties. Additionally, the correlation of antioxidant capacity with melatonin and total phenolic content was researched.

MATERIAL AND METHOD

The plant materials of the study were *Lycium barbarum* (goji berry/wolfberry), *Morus nigra* (black mulberry), *Morus alba* (white mulberry), *Rubus fructicous* L. (blackberry), *Morus rubra* (purple mulberry) and Bursa Black mulberry. *Lycium barbarum* is a deciduous woody shrub, growing 1–3 metres high. It's fruit is a bright orange-red and ellipsoid berry. Fruits of *Morus nigra* species are dark-colored, sweet-sour; those belonging to the *Morus alba* species are white and purple, sweet and low acid; and those belonging to *Morus rubra* species are dark-colored, sweet, high in dry matter, and low acidity. Bursa Black mulberry has black, sweet, and low acidity fruits. *Rubus fruticosus* is a European blackberry species in the genus *Rubus* in the *Roseceae* family. Fruit samples grown in Malatya ecology were used for the study. The samples were stored in a deepfreeze until analysis.

In this study, chemicals with analytical purity were used. For extraction and determination of spectrophotometric assays, gallic acid, Folin-Ciocalteu reagent, 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), melatonin from Sigma-Aldrich (Germany); Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was obtained from Acros Organics (USA); methanol, hydrochloric acid, sodium carbonate, potassium persulfate from Merck (Germany) were purchased.

Extraction Procedure and Spectrophotometric Analyses

The homogenized fruit samples were extracted in the solvent mixture of methanol: water: HCI (70: 29.9: 0.1 v/v/v) and then was filtered through a 0.45 µm PVDF (polyvinylidene difluoride) filter. This filtrate was used in all analyzes. The total phenolic content (TPC) was determined by the Folin-Ciocalteu method (Hwang, Shue, & Chang, 2001), and the results were expressed as mg gallic acid (GAE)/100 g_{FW} (fresh weight). UV/VIS Spectrophotometer (Shimadzu 2000S Model, Japan) was used for detection of TPC in berry fruit samples. To determine the antioxidant capacity, 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) radical scavenging tests were performed. DPPH radical scavenging test was performed by using the method of Yen & Hung (2000). ABTS radical scavenging test was performed by using the method of Rajurkar & Hande (2011). Results were compared with a standard curve prepared with Trolox, and expressed as mg Trolox (TE)/100 g_{FW}.

Analysis of Melatonin

Melatonin extracted from the fruit samples was identified by HPLC, equipped with Shimadzu DGU-20A5 model vacuum degasser and Shimadzu 20 ADXR solvent pump. Separations were performed using a Welch Welchrom C18 5 µm reversed-phase column (250 mm × 4.6 mm). Detection was performed with a Shimadzu RF-20A fluorescence detector. A solvent mixture consisting of water: methanol: acetic acid was used as the mobile phase. The melatonin content was determined according to its calibration curve (Figure 1) and expressed as ng/g_{FW}.

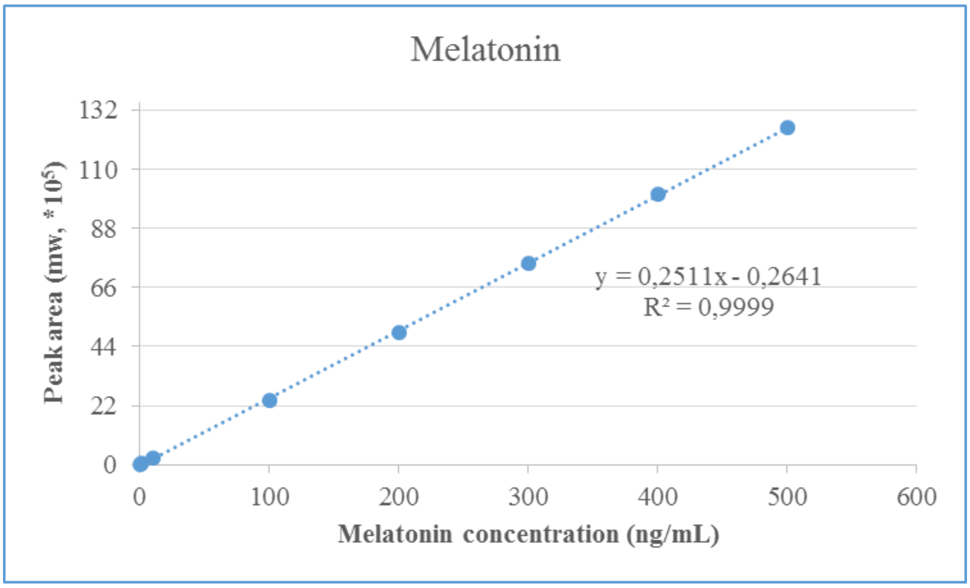

Figure 1. Calibration Curve of Melatonin Standard

Statistical Analysis

Analyses were performed with three replicates. All data were subjected to variance analyses. Significant differences among applications were determined according to LSD multiple comparison test at $p < 0.05$. In addition, correlation coefficients between results of the analysis were calculated.

RESULTS AND DISCUSSION

The results of melatonin, TPC, and antioxidant capacity to DPPH and ABTS tests in fruit samples are summarized in Table 1 and Figure 2-5. There were significant differences ($p < 0.05$) observed between berry fruits on the investigated parameters. Melatonin content was analyzed by HPLC-FD in 6 berry fruits. The amounts of this compound in fruit samples ranged from 123.44 to 1600.48 ng/g $_{FW}$. BF1 (goji berry/wolfberry) had attracted attention for its high melatonin content (Table1, Figure 2). It was observed that the TPC, DPPH and ABTS test results of the samples varied between 90.38 and 276.83 mg GAE/100 g_{FW}, 143.19 and 452.38 mg TE/100 g_{FW}, and 140.41 and 555,73 mg TE/100 g_{FW}, respectively. BF6 (blackberry) was the prominent berry with total phenolic content and antioxidant capacity to DPPH and ABTS tests (Table 1, Figure 3-5). However, the same sample was the fruit with the lowest melatonin content (Table 1, Figure 2). BF3 (white mulberry) was a berry that showed low antioxidant capacity in parallel with its low total phenolic content (Table 1, Figure 3-5).

Table 1. Results of Melatonin, TPC and Antioxidant Capacacity in Berry Fruits Extracts; Data are Expressed as mean±SD (n = 3); Means in the Same Column Bearing Different Letters are Significantly Different ($p < 0.05$)

	Melatonin (ng/g_{FW})	TPC (mg GAE/100 g_{FW})	DPPH (mg TE/100 g_{FW})	ABTS (mg TE/100 g_{FW})
BF1	1600.48±23.66^{a}	163.76±0.60^{c}	178.24±14.31^{d}	349.97±1.83^{c}
BF2	178.70±0.57^{c}	188.13±2.05^{b}	368.91±11.75^{c}	347.26±1.52^{c}
BF3	183.29±6.27^{c}	90.38±0.37^{e}	143.19±6.04^{e}	140.41±2.75^{e}
BF4	177.91±27.51^{c}	123.70±11.05^{d}	167.60±6.20^{d}	181.03±4.59^{d}
BF5	233.86±7.20^{c}	185.32±1.74^{b}	406.44±7.04^{b}	411.89±3.60^{b}
BF6	123.44±2.05^{d}	276.83±8.46^{a}	452.38±2.33^{a}	555.73±13.12^{a}

(BF1: Goji berry/wolfberry, BF2: Black mulberry, BF3: White mulberry, BF4: Bursa black mulberry, BF5: Blackberry, BF6: Purple mulberry)

Melatonin is a natural component in low amounts in food. Researchers were identified melatonin in the different parts of plants including fruits, roots, stems, leaves, seeds and flowers. Chen et al. (2003) reported that the melatonin content in white mulberry leaf and dried goji berry was measured to 1510 and 530 ng/g, respectively. In another study, the melatonin amount in the seeds of goji berry was determined as 103 ng/g (Manchester et al., 2000). These data differ from our result (1600.48 ng/g). The fact that we used fresh whole fruit in our study may be a reason for such a difference. It has been reported that goji berry fruit contains 10-fold higher more melatonin than goji berry seeds (Manchester et al., 2000). Melatonin is present in both the seed and pulp of fruits. Environmental conditions and oxidative stress may affect the pulp, whereas the seeds within the fruits are in a dormant state and thus are less exposed to the environmental conditions (Zohar et al., 2011). Fruit pulp that is more exposed to stress conditions can produce higher levels of melatonin. In the literature, the melatonin content of leaves generally in mulberry cultivars was investigated. The amount of melatonin in the leaves varies between 40.7 and 1510 ng/g (Chen et al., 2003; Pothinuch & Tongchitpakdee, 2011; Zohar et al., 2011). No data about the melatonin content of fruit samples were found. In a study conducted on different berry species, it was reported that the amount of melatonin in blackberry was 21 pg/g, which is quite low compared to the result of our study (Kolar & Malbeck, 2009). The melatonin concentrations in horticultural crops are closely influenced by factors such as species, varieties, growing environment, cultivated methods, harvesting time and extraction methods. Additionally, climate and environmental factors have significant effects on the melatonin concentrations of horticultural crops.

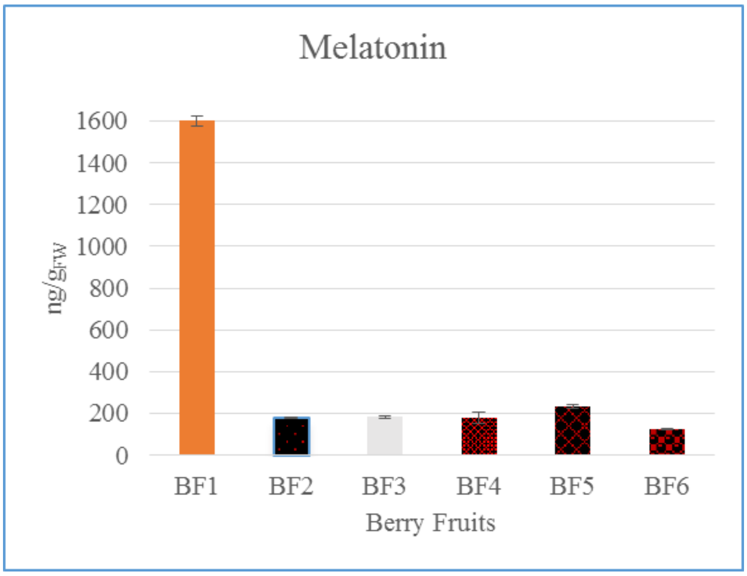

Figure 2. Melatonin Levels of Berry Fruits Extracts

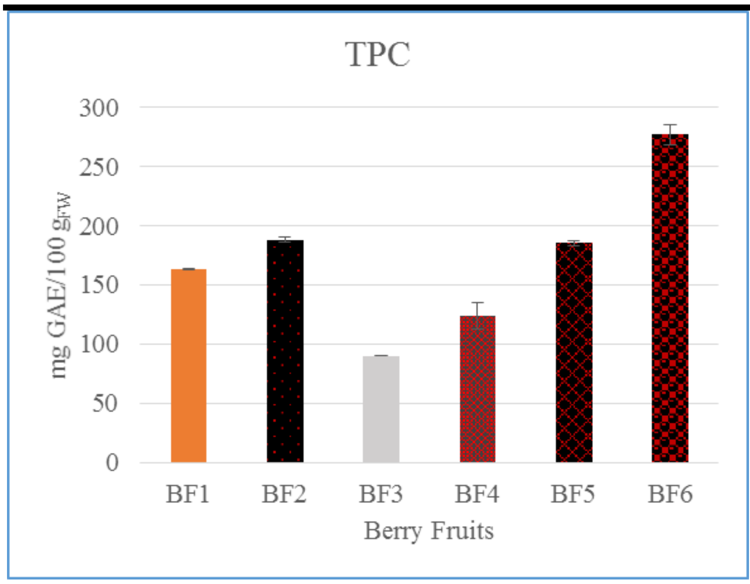

Figure 3. Total Phenolic Contents of Berry Fruits Extracts

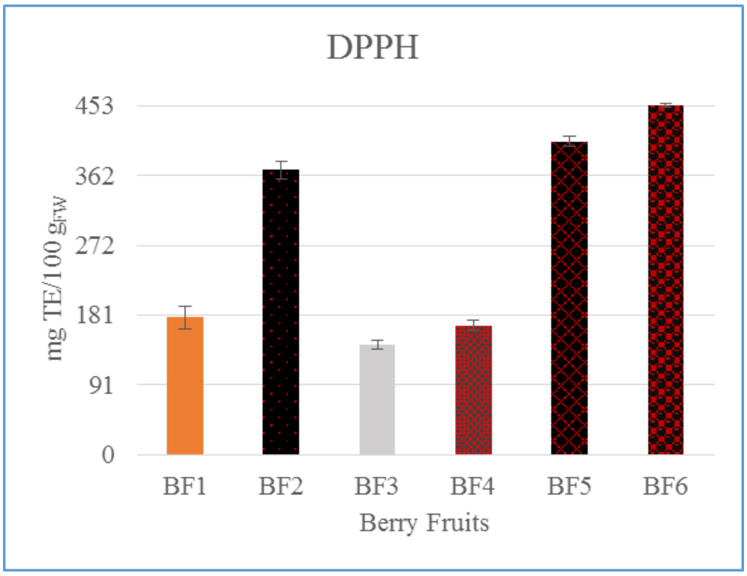

Figure 4. Antioxidant Capacity to DPPH Radical Scavenging Tests of Berry Fruits Extracts

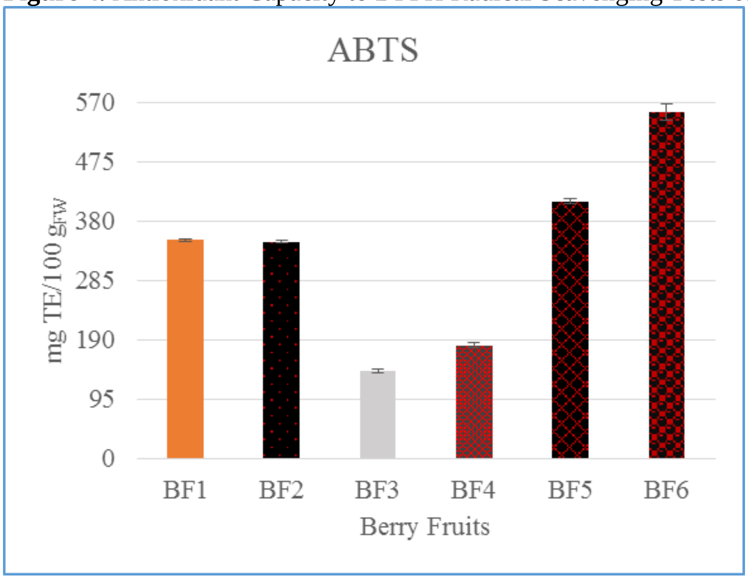

Figure 5. Antioxidant Capacity to ABTS Radical Scavenging Tests of Berry Fruits Extracts

It was observed that the antioxidant capacity was closely related to the total phenolic content of the fruit samples. Goji berry with high melatonin content showed low antioxidant capacity. BF6 (purple mulberry) with the lowest melatonin had the highest antioxidant capacity as the fruit with the highest total phenolic content. Although this result is surprising, it was stated that melatonin showed lower antioxidant capacity compared to phenolic compounds. (Aguilera et al., 2015).

The extracts from all mulberry fruits were tested with ABTS and DPPH to evaluate antioxidant capacity. The extracts from the fruits of mulberry cultivars showed antioxidant capacity between 140.41 and 555.73 mg TE/100 g_{FW} according to both tests. BF6 (purple mulberry) from mulberry cultivars had high antioxidant capacity, whereas BF3 (white mulberry) showed low antioxidant capacity. A similar situation exists for the total phenolic results of mulberry cultivars. In previous studies, total phenolic content and antioxidant capacity values of fruits containing *Morus alba*, *Morus nigra*, and *Morus rubra* varieties ranged between 104.8-4300.2 mg GAE/100 g and 6.17-7475.6 mg TE/100 g, respectively (Bae et al., 2015; Bae & Suh, 2007; Chen, Li, Bao, & Gowd, 2017; Ercisli et al., 2010; Gündeşli, Korkmaz, & Okatan, 2019; Kamiloğlu, Serali, Unal, & Capanoglu, 2012; Negro, Aprile, Bellis, & Miceli, 2019; Özgen, Serçe, & Kaya, 2009). The results of our study are in agreement with the data stated in the literature.

Antioxidant capacity and total phenolic values of BF5 (blackberry) were previously found between 28-432 mg TE/100 g and 48.9-690.2 mg GAE/100 g, respectively (Gündeşli et al., 2019; Huang, Zhang, Liu, & Li, 2012; Okatan, 2020). Zorzi et al. (2020) found that the antioxidant capacity of goji berry was 118 mg /100 mL. In another study using different extraction solvents, it was reported that the total phenolic content of goji berry varied between 9.28 and 174.27 mg GAE/100 g (Ionica, Nour, & Trandafir, 2012). Mocan et al. (2019) examined the antioxidant capacity of goji berry depending on different homogenization techniques, geographical origin and variety and determined that the results ranged from 218 to 6358 mg TE/100 g. Although the results of the current study are consistent with the literature data, there is variation in the data. Factors such as different ecological conditions, varieties, extraction methods and determination methods are the reason for this variation.

The analyses revealed a significant positive correlation between the TPC with DPPH and ABTS ($r=0.8730$, $r=0.9716$, $p < 0.001$). Similarly, a positive correlation was found between DPPH and ABTS ($r=0.8675$, $p < 0.001$). The melatonin content wasn't correlated with TPC, DPPH and ABTS (Tablo 2).

Table 2. Correlation Coefficients Among the Assessed Variables Obtained From Pearson's Correlation Test

	Melatonin	**TPC**	**DPPH**	**ABTS**
Melatonin	1.0000	-0.0879	-0.3907	0.0406
TPC		1.0000	0.8730*	0.9716*
DPPH			1.0000	0.8675*
ABTS				1.0000

*p < 0.05

CONCLUSION

In this study, some chemical properties of six berry species were determined. Goji berry have the highest melatonin content. Purple mulberry was the prominent variety with both total phenolic content and antioxidant capacity. Further studies, both in vitro and in vivo, are needed to evaluate the health potential of these two cultivars.

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