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DETERMINATION OF THE TOTAL PHENOLIC AND ANTHOCYANIN CONTENTS, AS WELL AS THE TOTAL ANTIOXIDANT CAPACITY, OF BLACK WOLFBERRY (*Lycium ruthenicum*) FRUITS

DETEKCIJA UKUPNOG FENOLA, UKUPNOG ANTOCIJANINA, UKUPNOG ANTIOKSIDANTNOG KAPACITA CRNOG GOĐİJA (Licium ruthenicum)

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ABSTRACT

The black wolfberry (Lycium ruthenicum) belongs to the Solanaceae family. The genus Lycium comprises 87 recognized species and is distributed in arid and semi-arid regions of temperate to subtropical zones in the world. At present, China is the greatest global producer and supplier of wolfberry products. The goji berry has recently became one of the most popular fruits in Turkey due to its health-beneficial compounds such as phenolic compounds (phenolic acids and flavonoids), carotenoids, tocopherol, ascorbic acid and antioxidant properties. Lycium fruits have been used as a remedy since ancient times in Asian countries, especially in China, for their emmenagogue, diuretic, antipyretic, tonic, aphrodisiac, hypnotic, and hepatoprotective effects. The purpose of this paper is to determine the biochemical characteristics (namely total phenolic and anthocyanin contents, as well as the total antioxidant capacity) of black wolfberries grown under the Aksaray ecological conditions.

Keywords: *antioxidants, total phenol, total anthocyanin, black goji berry.*

REZIME

Crne gođi bobice (Lycium ruthenicum) pripadaju porodici Solanaceae porodici. Rod Lycium sadrži 87 prepoznatih vrsta i rasprostranjen je u sušnim i polu-sušnim regionima u umerenim do suptropskim zonama u svetu. Kina je trenutno najveći isporučila ovog voća iz svojih voćnjaka u svetu. U ovoj zemlji se uzgajaju komercijalne količine. Nedavno je u Turskoj gođi bobica postala jedno od popularnih voća zbog korisnih jedinjenja za zdravlje, posebno fenolnih jedinjenja (fenolne kiseline i flavonoidi), karotenoida, tokoferola, askorbinske kiseline i antioksidativnih svojstava. Plodovi gođija se od davnina koriste kao lek u azijskim zemljama, posebno u Kini zbog efekta emenagoga, diuretika, antipiretika, tonika, afrodizijaka, hipnotičara i hepatoprotektive. Cilj studije bio je da se otkriju biohemijske karakteristike kao što su ukupni fenol, ukupni antocijanin i ukupni antioksidativni kapacitet biljke crnog gođija, koja je uzgajana u ekološkim uslovima Aksarai u Turskoj.

Ključne reči: *antioksidanti, ukupni fenol, ukupni antocijanin, bobica crnog gođija.*

INTRODUCTION

The fruit of *Lycium* spp., also called Boxthorn, Matrimony vine, wolfberry or goji berry, is well-known for its anti-ageing and eyesight improving effects. In China, it is used both as a medication in Chinese medicine and food in Chinese cuisine. Many researchers have observed that the *Lycium barbarum* fruits are rich in compounds which can heal and restore the entire human body, from the skin and the liver to the brain and the eyes. For this reason, the consumption of *Lycium barbarum* facilitates the Yin/Yang balance in the human body necessary for the protection from any possible age-associated diseases. *Lycium barbarum* produces red-coloured fruits called Fructus lycii, Gouqizi, goji berries or wolfberries, which have been used as a traditional Chinese herbal medicine for thousands of years (*Xin et al. 2011*). *Lycium barbarum* species are closely related to *Lycium chinense*. Both are medicinal plants native to China, but are also commonly found in Korea, Japan, and other Asian countries (*Zhong et al. 2013*). The wolfberry is very important to human health on account of its versatile pharmacological functions, anti-ageing properties, high antioxidant and phenolic compound ingredients, and immune system boosting properties. Furthermore, it has eyesight improving and anti-ageing features, as well as anti-tumour effects (*Yu et al., 2007; Amagase et al., 2009; L. et al., 2004; K.T. et al., 2005*). Phenolics comprise plant flavonoids which have been found to exert effects on radical scavenging, antioxidation, and cancer prevention. Flavonoids are important active compounds present in the leaves of L. barbarum (*Perez et al., 2009; Bystrom et al., 2009*). *L. ruthenicum* is another traditional Chinese medicinal plant used for the treatment of heart diseases, unnatural menstrual cycles and menopause (*Zheng et al., 2011*). A study conducted in China established that the functional components of *L. ruthenicum* black fruits primarily consist of anthocyanins, essential oils and polysaccharides (*Zheng et al., 2011*). Over the past years, wolfberries and wolfberry juice have been sold as healthy food products in markets around the world with increased popularity. *Lycium barbarum* and *L. chinense* are immensely important to the wolfberry production in East Asia and China. The Lycii fruits, root bark (cortex *Lycii radicis*) and leaves have been used as dried products, whereas offshoots are used in traditional Chinese medicine (*Potterat, 2010*). *Lycium barbarum* L., a traditional Chinese medicinal herb, has been widely used as a significant functional component of healthy foods for its life-sustaining biological activities (namely the preservation of age-related macular deterioration, the inhibition of cancer cell proliferation and the improvement of immune response (*L. et al., 2004*; *K.T. et al., 2005*)). These beneficial effects have been associated with different functional components such as polysaccharides, flavonoids, phenolic acids and carotenoids. Wolfberries provide various health benefits such as the nourishment of the yin, the strengthening of the liver and the kidneys, and the blood sustenance (*Luo et al. 2004*, *Burke et al. 2005*). In recent studies, it has been discovered that wolfberry flavonoids preserve the blood cells and mitochondria against oxidative damages (*Luo et al. 2004*). Furthermore, wolfberries have been found to posses interesting antioxidant, immune-enhancing, radioprotective, anti-ageing, and other health benefits. Wolfberries are very rich in nutrients with high antioxidant capacity, a property for which they have been included in the novel category of “superfruits”. Antioxidant compounds can be water-soluble, lipid-soluble, insoluble or bound to cell walls. The most utilised solvents for determining the radical scavenging activity using the DPPH method are methanol and ethanol. The concentration of the DPPH working solution in discussed methods ranges from 0.05 mM to 1.5 M, but a concentration of 0.10 mM is mostly used. Different DPPH concentrations lead to substantial differences in the ratio between the sample and reagent volumes. Different ratios (3:1, 1:1, 1:7.5) have been reported in the literature. The difference in the reaction of radical scavenging activity between DPPH solutions and the sample examined varied from 1 minute to 240 minutes. The determination of radical scavenging activity by DPPH is effectuated under different wavelengths such as 492 nm, 515 nm, 517 nm, 518 nm, 520 nm and 540 nm. The radical scavenging activity could be calculated by using different standard solutions such as vitamin C (L-ascorbic acid), Trolox, vitamin E (α-tocopherol), BHT and BHE. Antioxidants are substances which significantly delay or prevent the substrate oxidation (*Halliwell and Gutteridge, 1989*). The most abundant antioxidants in fruits and vegetables are polyphenols and vitamins, especially vitamin C. Vitamins A, B and E and carotenoids are present to a lesser extent in some fruits. These polyphenols, most of which are flavonoids, are present mainly in ester and glycoside forms (*Lim et al., 2007*). To determine the number and antioxidant capacity of various substances with antioxidant features, the initial procedure is to extract them from the sample using a solvent. Water, aqueous mixtures of ethanol, methanol, acetonitrile, acetone and hydrochloric acid are commonly used to extract plants substances (*Sun and Ho, 2005*). Furthermore, antioxidants are significant shelf life extenders for lipids and lipid-including foods. Increased attention has been recently devoted to the use of natural antioxidants, especially of plant origin insted of synthetic antioxidants, such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT). Moreover, organic acids sugars and phenolic compounds, total antioxidant capacity and anthocyanins are regarded as main quality factors by both consumers and the food industry (*Radovanović et al.; 2011; Oz et al., 2017*).

Wolfberries contain high amounts of antioxidants, carotenoids, vitamin A and zeaxanthin. They are rich in vitamins B and C, as well as polysaccharides. These fruits have a high protein content (10 %) and provide 18 various amino acids, of which 8 are essential (*Ionica, et al., 2012*). *Dong et al. (2012)* carried out a study on the total flavonoid content in wolfberries grown in various regions in China. Their results indicate that the highest amount of flavonoids (2.97 %) and the most intense antioxidant activity of wolfberries were recorded in wolfberries produced in the Zhongning region. *Yu et al. (2006)* demonstrated that *L. barbarum* extracts can protect neurons against ß-amyloid (Aß) peptide-induced apoptosis. Using a reducing agent, dithiothreitol (DTT), they determined that LBG exhibits cytoprotective effects for reducing stress by lowering the DTT-induced and caspase-3 activity. These results suggest that AA-2βG is an important antioxidant component of wolfberry fruits, which may share similar yet distinct antioxidant properties with AA (L-Ascorbic acid). The present study is aimed at revealing the biochemical properties (namely the total phenolic and anthocyanin contents, as well as the total antioxidant capacity) of black wolfberries grown under the Aksaray ecological conditions.

MATERIAL AND METHOD

This study was carried out at a wolfberry plantation (belonging to HZR Fidan AS) in the Village of Kargın, the Aksaray province, Turkey. Wolfberries were planted at a spacing of 3.5 x 1.5 m in the June of 2014, using the wire training system and a random block design in a four-year-old wolfberry garden. Fruit samples were randomly selected from a total of 20 plants.

Total Phenolic Content and Total Antioxidant Capacity

The total phenolic content of goji berry samples was determined using the modifying spectrophotometric Folin-Ciocalteu’s method developed by *Spanos and Wrolstad* (1990). For this purpose, a volume of 10 ml of methanolic extract was used for 1 g of homogenized goji berries. The values obtained were expressed as the mg gallic acid equivalent in a 100 g extract (mgGAE/100 g).

In this study, the antioxidant capacity of the goji berry samples was determined using the DPPH (2.2-diphenyl-1-picrylhydrazyl) radical-scavenging method (according to the method of *Brand-Williams et al. (1995*) and the modified method of *Duarte-Almeida et al. (2006)).* A 50 µL aliquot of the extract previously diluted and 250 µL of DPPH (0.5 mM) were mixed. After 20 minutes, the absorbance was measured at 517 nm using a Microplate Spectrophotometer (Benchmark Plus, BioRad, Hercules, CA). The control consisted of a methanolic solution of Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) at different concentrations. The antioxidant capacity was expressed as the µmol Trolox equivalents of the g^{-1} sample in fresh weight (FW). The radical scavenging activity was determined by measuring the absorbance at 517 nm using the spectrophotometer and expressed as RA (%) = (1–absorbance at 517 nm after 30 min/absorbance at 517 nm after 0 min) x 100.

Total Anthocyanin Content

The total anthocyanin content (TAC) of the goji berry samples was estimated by the pH differential method (*Giusti and Wrolstad, 2001*) with slight modifications. The fruits of *Lycium ruthenicum* were homogenized in 10 mL methanol containing 1 % HCl for two minutes, kept overnight, and filtered using the Whatman No. 2 filter paper. Two extracts were prepared: one with a potassium chloride buffer (pH 1.0) (1.86 g KCl in 1 L of distilled water) and the other with a sodium acetate buffer (pH 4.5) (54.43 g $CH_3CO_2Na_3H_2O$ in 1 L of distilled water). The absorbance of the extracts was measured at 510 nm and 700 nm (SQ2800, Unico UV visible Spectrophotometer, USA) after 15 min of incubation at room temperature. The total anthocyanin content goji berries was determined on the basis of the molar absorptivity of cyaniding-3-glucoside and expressed as the cyaniding-3-glucoside equivalent. The anthocyanin content obtained was calculated using the coefficient of molar absorptivity (Total Anthocyanins (mg/100 g) = (A × MW × DF × 10000)/ε × l (A = Absorbance difference, MW = Molecular Weight (MW : 449.2), DF = Dilution Factor, ε = molar extinction coefficient (ε : 26900, l = pathlength (1 cm)).

RESULT AND DISCUSSION

Fruits rich in phenolic compounds are gaining prominence in the food industry due to their ability to hinder the oxidative degradation of food lipids and enhance the quality and nutritive value of food. Over the past decades, naturally occurring antioxidants have been supplanting synthetic antioxidants (implicated in carcinogenicity) in the food and medical industries. The results obtained for the total phenolic and anthocyanin contents, as well as the total antioxidant caopacity, of fresh and dried fruits of *Lycium ruthenicum* are given in Table 1. As seen in Table 1, the highest total phenolic content was determined in dried goji berries (1039.48 mg/100g), and was found to be higher than that of fresh fruits (618.87 mg/100g). In a previous study, *Kosar et al. (2003)* examined the total phenolic compounds of *Lycium ruthenicum*. The authors determined that the total phenolin content ranged between 31.97±0.06 and 125.19±0.04 $mg_{GAE}/g_{extract}$. In another study, *Ionica et al. (2012)* noted that the extraction with 80 % alcohol, as well as with 2 % hydrochloric acid, has resulted in the highest values of the phenolic content and were consistent with the results previously reported by *Kayashima and Katayama (2002*). The total phenolic compound values determined were higher compared to the Kosar's results. This may be accounted for by the use of different extraction solvents and goji berry plants grown under various climate and soil conditions. Consistent with our results, *İslam et al. (2017*) reported an average value of 4 black goji berry samples of 8.33 mg GAE/g, implying that black goji berries have the total phenolic content 2.6 times higher than that of red goji berries.

The total antioxidant capacity of *Lycium ruthenicum* goji berry fruits was 87.17 µmTrolox/100 g in dried samples and 87.68 µmTrolox/100 g in fresh samples. Furthermore, fresh *Lycium ruthenicum* fruits exhibited a bit higher antioxidant activity than that of dried fruits. İslam et al (2017), reported an average value of 4 black goji berry samples of 34.28 µmol TE/g, which was 2 times higher than that recorded in red goji berries. The results obtained in the present study proved even higher.

The highest total anthocyanin content was determined in dried goji berry fruits (174.41 mg/g). Fresh fruits (72.03 mg/g) exhibited the lowest total anthocyanin content. *Kosar et al. (2003*) determined that the anthocyanin content of *Lycium ruthenicum* fruits ranged between 0.894mg/g and 2.193mg/g using the cyanidin-3 glucoside by HPLC technique. *İslam et al. (2017)* reported an average monomeric anthocyanin content of black goji berry samples of 72.86 mg MAC/g, which was 30.4 times higher than that of red goji berries. The results obtained in the present study proved higher than the ones previously reported in the literature. This may be accounted for by different climate and soil conditions, as well as different goji berry cultivars used as experimental material.

Table 1. Total phenolic and anthocyanin contents, as well as the total antioxidant capacity, of fresh and dried Lycium ruthenicum fruits

Samples	Total Phenolic Content (mg/100g)	Total Antioxidant Capacity DPPH (µmTrolox/g)	Total Monomeric Anthocyanin (mg/g)
Fresh fruits	618.87±25.36	87.68±1.25	72.03±5.56
Dried fruits	1039.48±42.13	87.17±2.33	174.41±12.30

CONCLUSION

The results obtained in the present study indicate that dried fruits of *Lycium ruthenicum* have higher total phenolic and monomeric anthocyanin contents than those of fresh *Lycium ruthenicum* fruits. Moreover, the total antioxidant capacities of both fresh and dried *Lycium ruthenicum* fruits were found to be approximately the same.

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