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Research Article

Evaluation of *Rubus caesius* L. fruit different maturity stages on phytochemical properties and antioxidant activity

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Abstract

In this article, we have studied the changes in phytochemical contents and antioxidant properties of blackberry (*Rubus caesius* L.) fruits naturally grown in Battalgazi district of Malatya province during the ripening process. It was determined the total phenolic (23.82-151.36 mg GAE/g extract), total flavonoid (5.65-25.72 mg RE/g extract), and total flavonol (1.30-5.40 mg QUE/g extract) contents of twelve extracts prepared by six different solvents. According to these results, it was concluded that phytochemical contents were higher in unripe fruit extracts than the ripe fruits. The reducing power, chelating capacity of metal ions and antioxidant activity of each extract were determined using the DPPH free radical scavenging method. The working conditions in which the antioxidant activity values were best obtained by the DPPH method were explained according to each extract extraction method and it was seen that the best result was the acidified water extract of the unripe fruit (IC_{50} 27.24 ± 0.00 µg/mL). The results showed that *R. caesius* L. can be consumed as a natural antioxidant due to its antioxidant properties. Supporting these results with *in vivo* studies will also clarify the health promoting properties of antioxidant activity of *R. caesisus* fruit.

1. Introduction

The results of many epidemiological studies conducted to date have shown that long-term and regular consumption of foods rich in plant polyphenols can protect against the formation of acute and chronic diseases, especially cancer [1–3]. It has been understood that the endogenous defense system of the body should be supported by antioxidant compounds to be taken with a regular and balanced diet [4, 5]. For this reason, it is important to intake antioxidant rich diet. On the other hand, synthetic polyphenolic antioxidants which butylated hydroxytoluene, octyl gallate, dodecyl gallate, propyl gallate, tert-butyl hydroquinone, butylated hydroxyanisole, alpha-, gamma- and delta-tocopherol have been widely used as food additives [6, 7] but several reports have indicated high doses of synthetic antioxidant exert negative health influences such as skin allergies, DNA damage, DNA cleavage, gastrointestinal tract problems [6, 8], so the research of natural antioxidants from fruit and vegetable has increasing attention. Fruits such as berries contain a variety of compounds with antioxidant activity, including phenolic acids, flavonoids, vitamin C, anthocyanins, carotenoids, tannins, flavonols and flavanols [9].

The *R. caesius*, Europan dewberry together comprising approximately 700 species of the *Rubus*

genus belonging to the Rosaceae family [10]. The dewberry is a perennial shrub reaching between 50-200 cm in height [10-12] and this species grows on basic soils [31]. *R. caesius* L. is native to Europe but is also widely distributed in many countries worldwide such as Russia, the United States, Turkey, Italy, Spain [11-14].

It was noted that Rubus species are widely used in traditional and folk medicine in different regions of the world, as they show a wide distribution area in the world. In many parts of the world, the use of roots, leaves and fruits of Rubus species as antispasmodic, reducing menstrual and labor pains, healing wounds, diabetes, asthma, hemorrhoids, cholagogue, depurative, constipation and astringent, diarrhea, tonsillitis, allergic rhinitis is recorded in the literature. [10, 15-17]. Studies have shown that Rubus species are rich in antioxidant compounds. [10,17] and this plant has shown a variety of pharmacological activities like antioxidant [18, 19], anticancer, anti-inflammatory, antimicrobial, antibacterial [12], antidiabetic [11], antitumor [16]. According to the survey of the literature present a lot of data about antioxidant activities and chemical composition of the genus *Rubus*, while there are only a few data of the *Rubus caesius*. In addition, no research has been reported on the antioxidant activity and phytochemical content of unripe blackberries in the literature. This study was carried out to investigate the changes in the phytochemical and antioxidant properties of *Rubus caesius* L. fruits grown naturally in Battalgazi district of Malatya (Turkey) during the ripening process and to form a source for future biological, nanotechnological, phytochemical and breeding studies.

2. Materials and methods

Fresh dewberry fruits were harvested in 2020 at different dates during ripening (July of unripe stage and August of the ripe stage) from Malatya, in Turkey. Dewberries were classified based on the color green fruit (unripe) red-purple fruit (ripe). The plant was identified at the University of İnönü by Professor Arabacı and voucher specimen (ZT1002) was prepared and put in the Pharmacy Faculty herbarium of the University. All samples were dried in air at room temperature. After drying, the dried samples were ground powder.

2.1. Preparation of the extracts

Fifteen grams of each powdered unripe and ripe *Rubus caesius* L. were extracted with 250 mL of methanol, ethanol, water, 0.1% acidified methanol, 0.1% acidified ethanol and 0.1% acidified water by using Soxhlet apparatus for 3 hours. Here, twelve kinds of dewberry extract were obtained as follows:

1. Methanol extract of unripe fruit (UDM 1)
2. Ethanol extract of unripe fruit (UDE 1)
3. Water extract of unripe fruit (UDW 1)
4. Acidified methanol extract of unripe fruit (UDM 2)
5. Acidified ethanol extract of unripe fruit (UDE 2)
6. Acidified water extract of unripe fruit (UDW 2)
7. Methanol extract of ripe fruit (RDM 1)
8. Ethanol extract of ripe fruit RDE 1)
9. Water extract of ripe fruit (RDW 1)
10. Acidified methanol extract of ripe fruit (RDM 2)
11. Acidified ethanol extract of ripe fruit (RDE 2)
12. Acidified water extract of ripe fruit (RDW 2)

The solvents of the extracts were evaporated at 40 °C in the evaporator, (Heidolph, Germany) and stock solutions were prepared by weighing the remaining solid matter. These stock solutions were stored in the refrigerator at +4 °C until analysis.

2.2. Determination of total phenolic content

Determination of total phenols (TPC) in the extracts was done using Folin-Ciocalteu assay [20]. 50 µL (1 mg/mL) extract was added to a test tube with 450 µL distilled water. Folin reagent (1.0 N, 250 µL) was added to the mixture. After five minutes at ambient temperature, 1250 µL of sodium carbonate (7.5%, w/v) was added and shaken vigorously. Further, the mixture was permitted to incubate for 120 min and the absorbance was measured using a Spectrophotometer at 765 nm. After then, the sample concentration was calculated from the gallic acid standard curve equation ($y=0.0095x + 0133$, $R^2=0.9988$) and the result was expressed as mg gallic acid equivalents per gram of extract (mg GAE/g extract).

2.3. Determination of total flavonoid content

The number of total flavonoids (TFC) was measured using aluminum chloride colorimetric assay [21]. 500

µL extract solution (1 mg/mL) was mixed in a test tube and 4500 µL distilled water and 300 µL of 5.0% sodium nitrite solution were added. Incubation at room temperature was done for 5 min, and then 300 µL aluminum chloride (10.0%, w/v) was added followed by 2000 µL NaOH (1.0 M) after a further 6 min. Finally, 2400 µL of distilled water was added. Rutin was used to prepare a standard calibration curve ($y=0.0065x + 0.0272$, $R^2=0.9970$), where y is the absorbance at 510 nm and x is the sample concentration in µg/mL. The content of total flavonoid in the extract was expressed as milligram rutin per gram of extract (mg RE/g extract).

2.4. Determination of total flavonol content

Total flavonol content (TFLC) was determined by applying slight modification to the method previously described by Kumaran and Karunakaran [22]. According to the method, 1 mL of extract (1mg/mL) was taken into different test tubes separately. 1.0 mL of 2.0% $AlCl_3$ and 3 mL of 5.0% $C_2H_3NaO_2$ solution were added to the test tubes. After gentle mixing, all test tubes were incubated at room temperature for 30 minutes. The absorbance was then determined using a spectrophotometer at a wavelength of 440 nm. The measurement was compared to a calibration curve of quercetin ($y=0.0161x + 0.049$, $R^2= 0.9976$) and final results were given as milligrams of quercetin equivalents (mg QUE/g extract).

2.5. Determination of DPPH radical scavenging activity

The free radical scavenging activities of the extracts were determined using the DPPH free radical [23]. For the DPPH• assay, 3.0 mL sample extract or standard (BHA, BHT and α-tocopherol) with varying concentrations (12.5, 25.0, 37.5, 62.50 and 125 µg/mL) were added to the 1 mL of DPPH• ethanolic solution (0.10 mM) in a test tube. The mixture was mixed thoroughly. After 30 minutes of incubation in the dark and at ambient temperature, the absorbance of the samples was measured at 517 nm. The analyses were performed in triplicate and the inhibition percentage of DPPH• discoloration was calculated using the following Eq.

$$\% \text{ Inhibition} = [(A_{control} - A_{sample})/A_{control}]x100$$

2.6. Reducing power

The method described by Oyaizu [24] was performed in the present study to determine the reducing power of extract. 1 mL of plant extract, 1 mL of 0.2 M phosphate buffer solution (pH 6.6) and 2.5 mL of 1% $K_3[Fe(CN)_6]$ solution were added to a test tube. Shake vigorously and incubate at 50 °C for 20 minutes. At the end of the period, 2.5 mL of 10% trichloroacetic acid solution was added and centrifuged at 6000 rpm for 10 min. After taking 1.25 mL from the solution, 1.25 mL of distilled water and 0.5 mL of 0.1% $FeCl_3$ were added. Finally, absorbance values were read at 700 nm.

2.7. Metal chelating activity

The chelating capacity of the extracts for Fe^{2+} ions was determined according to the method specified by Dinis et al. [25]. 3750 µL of extract at different concentrations (12.5-125 µg/mL) and 50 µL of 2.0 mM $FeCl_2$ were mixed. It was allowed to incubate for 10 min and then 200 µL of 5.0 mM ferrosine was added to start the reaction mixture. After mixing the solution, it was incubated for 20 minutes at room temperature and absorbance was measured at 562 nm. EDTA was used as a positive control in this assay system. The Fe^{2+} chelating activity of the dewberry extracts was calculated by the following equation:

$$\% \text{ Chelating Activity} = [(Abs_{control} - Abs_{sample})/Abs_{control}]x100$$

3. Results and discussion

Table 1 shows the percentage yields of extract of unripe and ripe dewberry fruit. The yield of ripe dewberry fruit extract was found to be the highest (22.02 %) by methanol extraction, followed by acidified methanol extraction (20.43 %) whereas, the lowest yield (1.39 %) was obtained from unripe dewberry fruit of ethanol extraction.

Table 1. Extraction yields (Y) of unripe and ripe dewberry in different solvent

Extracts	Y (%)	Extracts	Y (%)
UDM 1	3.49	RDM 1	22.02
UDE 1	1.39	RDE 1	4.87
UDW 1	2.61	RDW 1	19.72
UDM 2	2.66	RDM 2	20.43
UDE 2	2.62	RDE 2	5.14
UDW 2	2.05	RDE 2	17.56

Phenolic compounds like flavonoids, flavanols, phenolic acids and anthocyanins are the major determinant of antioxidant potentials in plants and they could be a natural source of antioxidants, anti-cancer and anti-mutagenic effects [26, 27]. The Folin-Ciocalteu method was chosen to the measure total phenolic content of different dewberry fruit extracts. Generally, the green dewberry fruit (unripe) had higher total phenolic content compared to the red dewberry fruit (ripe). Total phenolic content ranged from 23.82 ± 0.00 to 58.72 ± 0.00 mg GAE/g extract in ripe fruit, from 78.24 ± 0.00 to 151.36 ± 0.00 mg GAE/g extract in unripe fruit. As can be seen from Table 2, the highest TPC was showed in UDW 2, while the lowest was observed in RDE 2. Consequently, UDW 2 extract is expected to display powerful antioxidant activities. Sariburun and his co-workers, suggested that the phenolic content of the blackberry cultivars ranged from 2279.9 ± 13.0 to 2786.8 ± 21.9 mg GAE/g fresh weight [28] and Radovanović et al., [29] have found 7838.26 ± 1.64 mg GAE/kg in acidified methanol extract of wild blackberry.

Flavonoids comprise a wide range of phenolic compounds with known properties that include anti-inflammatory, anti-cancer, anti-mutagenic and antioxidant activities [30, 31]. The colorimetric method was chosen to the measure total flavonoid content of dewberry fruit extracts. The result of the total flavonoid content of the unripe and ripe dewberry fruit extracts is also presented in Table 2. Total flavonoid content ranged from 7.45 ± 0.02 to 25.75 ± 0.00 mg RE/g extract in unripe fruit, from 5.65 ± 0.01 to 9.60 ± 0.00 mg RE/g extract in ripe fruit. The results showed that the highest total flavonoid content was observed in the methanolic extract of unripe dewberry fruit, while the lowest total flavonoid content was obtained in the acidified water extract of ripe dewberry fruit. Caruso et al., [32] reported that the total flavonoid content of wild blackberry extract ranged between 1136 and 3174 mg quercetin/100 g fresh weight. Croge et al., [33] reported that the total flavonoid content of Tupy, Guarani, Xavante and Cherokee cultivars were between 30.44 and 47.33 mg quercetin/100 g fresh fruit.

Flavonols are a sub-group of flavonoids and the widest flavonols found in food are quercetin, myricetin and kaempferol [34, 35]. Dietary intake of flavonols is associated with many health benefits which include antioxidant, anti-inflammatory effects and reduced risk of vascular disease [35, 36]. The procedure reported by Kumaran and Karunakaran [22] was used to determine the total flavonol in the extracts. The total flavonol content varied from 1.68 ± 0.02 to 5.40 ± 0.00 mg QUE/g extract in unripe fruit, from 1.30 ± 0.01 to 3.42 ± 0.01 mg QUE/g extract in ripe fruit (Table 2). The highest flavonol content was determined in the methanolic extract of unripe dewberry fruit, while the lowest flavonol content was detected in the acidified water extract of the ripe dewberry fruit. Contents of flavonol decreased right along throughout ripening. Similarly, Acosta-Montoya et al., [37] reported a decrease in flavonol values for *Rubus adenotrichus* Schltdl. during maturing.

Table 2. Total phenol, total flavonoid and total flavonol contents and antioxidant activity of different unripe and ripe dewberry extracts

Extracts	TPC (mg GAE/g extract)	TFC (mg RE/g extract)	TFLC (mg QUE/g extract)
UDM 1	109.80 ± 0.01	25.75 ± 0.00	5.40 ± 0.00
UDE 1	103.94 ± 0.00	22.60 ± 0.00	4.91 ± 0.00
UDW 1	78.24 ± 0.00	9.92 ± 0.01	1.86 ± 0.00
UDM 2	105.88 ± 0.01	21.90 ± 0.00	4.66 ± 0.01
UDE 2	100.78 ± 0.02	18.45 ± 0.01	3.73 ± 0.00
UDW 2	151.36 ± 0.00	7.45 ± 0.02	1.68 ± 0.02
RDM 1	37.74 ± 0.00	9.60 ± 0.01	3.42 ± 0.01
RDE 1	25.20 ± 0.01	9.30 ± 0.00	2.42 ± 0.00
RDW 1	47.22 ± 0.00	6.60 ± 0.00	1.49 ± 0.01
RDM 2	28.22 ± 0.03	8.85 ± 0.01	3.29 ± 0.01
RDE 2	23.82 ± 0.00	8.00 ± 0.02	2.04 ± 0.01
RDW 2	58.72 ± 0.00	5.65 ± 0.01	1.30 ± 0.01

The antioxidant capacity of the plant extract is affected by a wide range of factors such as the composition of the extract and solvent type, and cannot be entirely utilized by one single method [38]. Owing to, in the present study, antioxidant activity was assessed by three assays which are propped up various mechanisms of antioxidant action. Fig. 1 exhibits variation in DPPH• scavenging activity of the dewberry extracts which ranged from 3.09 ± 0.00 to 83.16 ± 0.00%. UDW 2 exhibited the highest (83.16 ± 0.00%) activity followed by RDW 1 and RDW 2 (78.69 ± 0.00%) and UDM 1 (78.35 ± 0.00%), whereas RDE 2 showed the lowest activity (20.61 ± 0.00%) at the same

concentration of 125 µg/mL. To evaluate the antioxidant activity else, the IC_{50} for every extract was calculated (Table 3) and described as the concentration of extract inducing 50 % inhibition of absorbance, so a lower value would represent the greater antioxidant activity of the extracts. The results indicated that the highest active DPPH• scavenging activities of the acidified water extract were unripe dewberry (IC_{50} 27.24 ± 0.00 µg/mL), which less than that of BHA (21.74 ± 0.01 µg/mL), but higher than that of BHT (33.45 ± 0.01 µg/mL).

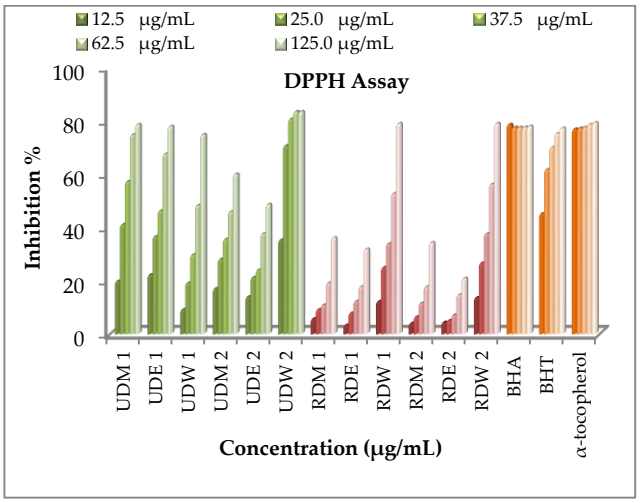

Figure. 1. DPPH free radical scavenging activity of blackberry extracts (%)

Table 3. Antioxidant activity of unripe and ripe Rubus *caesius* L. different extract

Extracts/ Standards	IC_{50} (µg/mL)	Extracts/ Standards	IC_{50} (µg/mL)
UDM 1	52.05 ± 0.01	RDM 1	175.48 ± 0.01
UDE 1	58.32 ± 0.00	RDE 1	194.44 ± 0.00
UDW 1	77.05 ± 0.00	RDW 1	70.24 ± 0.01
UDM 2	87.66 ± 0.01	RDM 2	182.73 ± 0.01
UDE 2	116.15 ± 0.01	RDE 2	296.18 ± 0.00
UDW 2	27.24 ± 0.01	RDE 2	67.58 ± 0.01
BHA	21.74 ± 0.01	α-tocopherol	1.55 ± 0.01
BHT	33.45 ± 0.01		

A comparative analysis on free radical scavenging activity of cultivated varieties of blackberry (Xavante and Cherokee) made by Denardin et al. [39] showed that DPPH• scavenging activity was found IC_{50} 44.70 mg/L and IC_{50} 78.25 mg/L, respectively. Moreover, the DPPH radical scavenging activity of dewberry in this study was much higher than that found by Keser et al. [40]. The antioxidant activities of unripe water and ethanolic blackberry fruit extracts on DPPH radicals were 38.13% and 49.82% at 100 µg/mL, respectively.

Ferric reducing the capacity of the plant extracts is usually related to the existence of reductones (antioxidant agents) that have been indicated to exert antioxidant activity of hydrogen donating and chain-breaking free radicals [41]. The data obtained (Table 4) in this study showed that both unripe and ripe extracts exhibit moderate reducing power capacity as compared with the standards. Keser et al. [40] indicated alterations in the phytochemical composition and antioxidant activity of *Rubus discolor* fruits for ripening. According to their results, unripe fruit extracts showed higher antioxidant activity using a reducing power assay than ripe fruit.

Table 4. Reducing power ability of different unripe and ripe dewberry extract

Extracts/ Standards	Reducing Power Ability (µg/mL, 700 nm)			
	5.88	14.70	29.41	44.11
	A	A	A	A
UDM 1	0.130±0.000	0.205±0.000	0.330±0.000	0.429±0.000
UDE 1	0.126±0.000	0.189±0.000	0.298±0.000	0.408±0.000
UDW 1	0.124±0.001	0.158±0.000	0.237±0.000	0.328±0.000
UDM 2	0.135±0.000	0.196±0.000	0.312±0.000	0.405±0.000
UDE 2	0.118±0.000	0.155±0.000	0.219±0.000	0.296±0.000
UDW 2	0.184±0.000	0.302±0.000	0.512±0.000	0.701±0.001
RDM 1	0.123±0.000	0.141±0.000	0.195±0.000	0.259±0.000
RDE 1	0.113±0.001	0.122±0.001	0.159±0.000	0.192±0.000
RDW 1	0.131±0.000	0.175±0.001	0.273±0.000	0.377±0.000
RDM 2	0.114±0.000	0.136±0.000	0.179±0.001	0.230±0.000
RDE 2	0.113±0.001	0.120±0.000	0.152±0.001	0.197±0.000
RDW 2	0.139±0.000	0.186±0.000	0.251±0.000	0.331±0.000
BHA	0.601±0.000	1.145±0.001	2.111±0.000	3.356±0.000
BHT	0.348±0.001	0.675±0.002	1.093±0.000	1.816±0.000
α-toco-pherol	0.268±0.000	0.521±0.000	0.877±0.000	1.029±0.001

Iron is an important dietary mineral for normal physiology, but an excess of it can lead to damage on a broad array of cellular structures since free iron can donate or accept and electron from next to molecules to bring about cellular injury and to create free radicals from reactive oxygen species [42, 43]. So, the chelating ability of ferrous ion of the plant extracts is an important mechanism for antioxidant activity. In the present study, we assessed the metal chelating

Table 5. The metal chelating ability of different unripe and ripe dewberry extrac

Extracts/ Standards	Metal Chelating Ability, Inhibition %				
	12.5 µg/mL	25.0 µg/mL	37.5 µg/mL	62.5 µg/mL	125.0 µg/mL
UDM 1	1.36±0.00	1.21±0.00	----	----	----
UDE 1	0.15±0.00	----	----	----	----
UDW 1	0.45±0.01	1.66±0.00	1.81±0.00	1.96±0.00	3.02±0.00
UDM 2	0.15±0.00	----	----	----	----
UDE 2	0.30±0.01	0.30±0.01	----	----	----
UDW 2	----	----	----	----	----
RDM 1	1.81±0.00	2.87±0.01	2.72±0.01	----	----
RDE 1	3.17±0.01	1.66±0.00	1.21±0.02	----	----
RDW 1	0.15±0.01	----	----	----	----
RDM 2	4.23±0.00	2.26±0.00	0.76±0.00	----	----
RDE 2	3.93±0.02	2.42±0.01	1.21±0.01	----	----
RDW 2	2.72±0.01	1.51±0.00	----	----	----
BHA	----	----	----	----	----
BHT	2.42±0.01	2.42±0.01	2.36±0.00	2.11±0.00	----
α-tocopherol	----	----	----	----	----
EDTA	10.12±0.01	53.32±0.00	90.18±0.00	91.39±0.01	93.95±0.01

ability of extracts of *R. caesius* and summarized Table 5. According to obtained data, RDM 2 and RDE 1 extracts showed maximum metal chelating ability at the concentration of 12.5 µg/mL compared to other extracts in each solvent. In addition, the ferrous chelating ability of acidified methanol extract of ripe dewberry was 1.7 times higher than BHT that using as a standard at the concentration of 12.5 µg/mL. Keser et al. [40] reported that ethanol and water extracts of unripe and ripe blackberry possess the potential metal chelating ability. Previous research on the 50% water-methanol extract from among the 20 plants including ripe *R. caesius* studied and dewberry showed moderated metal chelating activity (51.36%) [14]. This difference might be due to the climatic and soil differences, ripening period of fruit and used extraction solvent.

4. Conclusions

The results of this study, which investigated the chemical contents and biological activities of *Rubus caesius* L. fruit at different maturity stages, are considered promising. In later studies, the isolation, purification and structure elucidation of active compounds, especially in immature blackberries with bioactivity can be carried out. Obtaining the predicted results may provide possible economic value and form the basis for the identification or formulation of therapeutic or curative antioxidant preparations.

Authors' contributions

All authors contributed equally.

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Conflicts of interest

The authors have declared that no conflict of interest exists.

References

1. Cory, H.; Passarelli, S.; Szeto, J.; Tamez, M.; Mattei, J. The Role of Polyphenols in Human Health and Food Systems: A Mini-Review. Frontiers in Nutrition. 2018, 5(87), 1-9.
2. Pandey, K.B.; Rizvi, S.I. Plant Polyphenols as Dietary Antioxidants in Human Health and Disease. Oxid. Med. Cell. Longev. 2009, 2(5), 270-278.
3. Vauzour, D.; Rodriguez-Mateos, A.; Corona, G.; Oruna-Concha, M.J.; Spencer, J.P.E. Polyphenols and Human

Health: Prevention of Disease and Mechanisms of Action. Nutrients. 2010, 2(11), 1106-1131.
4. World Health Organization. Diet Nutrition and the Prevention of Chronic Diseases, Report of a Joint FAO/WHO Expert Consultation. 2003. Available online: whqlibdoc.who.int/trs/WHO_TRS_916.pdf (accessed on 20 November 2022).
5. World Health Organization. Fruit and Vegetables for Health: report of the Joint FAO/WHO Workshop on Fruit and Vegetables for Health. 2005. Available online: https://apps.who.int/iris/handle/10665/43143 (accessed on 20 November 2022).
6. Lourenço, S.C.; Moldão-Martins, M.; Alves V.D. Antioxidants of Natural Plant Origins: From Sources to Food Industry Applications. Molecules.2019, 24(22), 4132.
7. Nieva-Echevarría, B.; Manzanos, M.J.; Goicoechea, E.; Guillén, M.D. 2,6-Di-Tert-Butyl-Hydroxytoluene and Its Metabolites in Foods. Compr. Rev. Food Sci. Food Saf. 2015, 14(1), 67-80.
8. Dolatabadi, J.E.N.; Kashanian, S. A review on DNA interaction with synthetic phenolic food additives. Food Res. Int. 2010, 43(5), 1223-1230.
9. Skrovankova, S.; Sumczynski, D.; Mlcek, J.; Jurikova, T.; Sochor, J. Bioactive Compounds and Antioxidant Activity in Different Types of Berries. Int. J. Mol. Sci. 2015, 16(10), 24673-24706.
10. Grochowski, D.M.; Strawa, J.W.; Granica. S.; Tomczyk, M. Secondary Metabolites of *Rubus caesius* (Rosaceae). Biochem. Syst. Ecol. 2020, 92, 10411.
11. Schädler, V.; Dergatschewa, S. *Rubus caesius* L. Leaves: Pharmacognostic analysis and the study of hypoglycemic activity. Natl. J. of Physiol. Pharm. Pharmacol. 2017, 7(5), 501-508.
12. Rejewska, A.M.; Sikora, A.; Tomczykowa, M.; Tomczyk, M. Dewberry (*Rubus caesius* L., Rosaceae). Pharmacogn. Commun. 2013, 3(1), 55-57.
13. Ioncică, R; Bubulică, M.V.; Chirigiu, L.; Mogosanu G.D.; Popescu, R.; Popescu, H. Researches upon the Heavy Metals Content of *Rubus caesius* L. (*Rosaceae*). Curr. Health Sci. J. 2010, 36(1), 48-51.
14. Serteser, A.; Kargıoğlu, M.; Gök, V.; Bağcı, Y.; Ozcan, M.M; Arslan, D. Determination of antioxidant effects of some plant species wild growing in Turkey. Int. J. Food Sci. Nutr. 2008, 59(7-8), 643-651.
15. Gudej, J.; Tomzcyk, M. Determination of Flavonoids, Tannins and Ellagic Acid in Leaves from *Rubus* L. Species. Arch. Pharm. Res. 2004, 27(11), 1114-119.
16. Turker, A.U.; Yildirim, A.B.; Karakas, F.P. Antibacterial and Antitumor Activities of Some Wild Fruits Grown in Turkey. Biotechnol. Biotechnol. Equip. 2014, 26(1), 2765-2772.
17. Rocabado, G.O.; Bedoya, L.M.; Abad, M.J.; Bermejo, P. *Rubus*- A review of its Phytochemical and Pharmacological Profile. Nat. Prod. Commun. 2008, 3(3), 423-436.
18. Grochowski, D.M.; Paduch, R.; Wiater, A.; Dudek, A.; Pleszczynska, M.; Tomczykowa, M.; Granica, S.; Polak, P.; Tomczyk, M. In vitro Antiproliferative and Antioxidant Effects of Extracts from *Rubus caesius* Leaves and Their Quality Evaluation. Evid. Based Complement. Alternat. Med. 2016, 2016, 5698685.
19. Veličković, I.; Grujić, S.; Džamić, A.; Krivošej, Z.; Marin, P. D. *In vitro* Antioxidant Activity of Dewberry (*Rubus caesius* L. var. *Aquáticus* Weihe. & Ness.) Leaf Extracts. Arch. Biol. Sci. 2015, 67(4), 1323-1330.
20. Singleton, V.L.; Rossi, J.A. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am. J. Enol. Vitic. 1965, 16, 144-158.
21. Zhishen, J.; Mengcheng, T.; Jianming, W. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem. 1999, 64(4), 555-559.
22. Kumaran, A.; Karunakaran, R.J. *In vitro* antioxidant activities of methanol extracts of five *Phyllanthus* species from India. LWT 2007, 40, 344-352.
23. Blois, M. S. Antioxidant determinations by the use of a stable free radical. Nature 1958, 181, 1199-1200.
24. Oyaizu, M. Studies on product of browning reaction. Antioxidant activities of products of browning reaction prepared from glucosamine. Japanese J. Nutr. Diet. 1986, 44, 307-315.
25. Dinis, T.C.P.; Madeira, V.M.C.; Almeida, L.M. The action of phenolic derivatives (acetaminophen, salicylate, and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. Arch. Biochem. Biophys. 1994, 315(1), 161-169.
26. Kumar, N.; Goel, N. Phenolic acids; Natural versatile molecules with promising therapeutic applications. Biotechnol. Rep. 2019, 24, e00370.
27. Srivastava, A.; Greenspan, P. Hartle, D.K.; Hargrove, J.L.; Amarowicz, R.; Pegg, R.B. Antioxidant and anti-inflammatory activities of polyphenolics from Southeastern U.S. range blackberry cultivars. J. Agric. Food Chem. 2010, 58(10), 6102-6109.
28. Sariburun, E.; Şahin, S.; Demir, C.; Türkben, C.; Uylaşer, V. Phenolic content and antioxidant activity of raspberry and blackberry cultivars. J. Food Sci. 2010, 75(4), 328-335.
29. Radovanović, B.C.; Anđelković, S.M.; Radovanović, A.B.; Anđelković, M.Z. Antioxidant and antimicrobial activity of polyphenol extracts from wild berry fruits grown in Southeast Serbia. Trop. J. Pharm. Res. 2013, 12(5), 813-819.
30. Li, A.N.; Li, S.; Zhang, Y.J.; Xu, X.R.; Chen, Y.M.; Li, H.B.; Resources and biological activities of natural polyphenols. Nutrients. 2014, 6(12), 6020-6047.

31. Karak, P. Biological activities of flavonoids: An overview. Int. J. Pharm. Sci. Res. 2019, 10(4), 1567-1574.
32. Caruso, M.C.; Galgano, F.; Grippo, A.; Condelli, N.; Cairano, M.D.; Tolve, R. Assay of healthful properties of wild blackberry and elderberry fruits grown in Mediterranean area. J. Food Meas. Charact. 2019, 13, 1591-1598.
33. Croge, C.P.; Cuquel, F.L.; Pintro, P.T.M.; Biasi, L.A.; Bona, M.D.C. Antioxidant capacity and polyphenolic compounds of blackberries produced in different climates. HortScience. 2019, 54(12), 2209-2213.
34. Lavefve, L.; Howard, L.R.; Carbonero, F.; Berry polyphenols metabolism and impact on human gut microbiota and health. Food Funct. 2020, 11,45-65.
35. Dabeek, W.M.; Marra, V.M. Dietary Quercetin and Kaempferol: Bioavailability and Potential Cardiovascular-Related Bioactivity in Humans. Nutrients. 2019, 11(10), 2288.
36. Panche, A.N.; Diwan, A.D.; Chandra, S.R. Flavonoids: An overwiev. J. Nutr. Sci. 2016, 5: e47.
37. Acosta-Montoya, Ó.; Vaillant, F.; Cozzano, S.; Mertz, C.; Perez, A.M.; Castro, M.V. Phenolic content and antioxidant capacity of tropical highland blackberry (*Rubus adenotrichus* Schltdl.) during three edible maturity stages. Food Chem. 2010, 119(4), 1497-1501.
38. El Abed, N.; Guesmi, F.; Mejri, M.; Marzouki, M.N. (2014). Phytochemical screening and assessment of antioxidant, antibacterial and cytotoxicity activities of five Tunisian medicinal plants. Int. J. Pharma. Res. Bio Sci. 2014, 3(4), 770-789.
39. Denardin, C.C.; Hirsch, G.E.; da Rocha, R.F.; Vizzotto, M.; Henriques, A.T.; Moreira, J.C.F.; Guma, F.T.C.R.; Emanuelli T. Antioxidant capacity and bioactive compounds of four Brazilian native fruits. J. Food Drug Anal. 2015, 23(3), 387-398.
40. Keser, S.; Celik, S.; Turkoglu, S.; Yılmaz, Ö.; Turkoglu, I. Antioxidant properties of *Rubus discolor* L., extracts and protective effects of its flower extracts against hydrogen peroxide-induced oxidative stress in wistar rats. Turk. J. Pharm. Sci. 2015, 12(2), 187-206.
41. Abdolahi, N.; Soltani, A.; Mirzaali, A.; Soltani, S.; Balakheyli H.; Aghaei M. Antibacterial and antioxidant activities and phytochemical properties of *Punica granatum* flowers in Iran. Iran. J. Sci. Technol. Trans. A: Sci. 42: 1105-1110.
42. Sowndhararajan, K.; Kang, S.C. Free radical scavenging activity from different extracts of leaves of *Bauhinia vahlii* Wight & Arn. Saudi J. Biol. Sci. 2013, 20(4), 319-325.
43. Eid, R.; Arab, N.T.T.; Greenwood, M.T. Iron mediated toxicity and programmed cell death: A review and a re-examination of existing paradigms. Biochim. Biophys. Acta (BBA) Mol. Cell Res. 2017, 1864(2), 399-430.