Journal of Postharvest Technology
2021, **09**(3): 127-135
www.jpht.in

RESEARCH ARTICLE

Biochemical properties and antimicrobial and antioxidant activity of blackberry growing naturally in Kelkit valley

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Received: 15.04.2021 Accepted: 19.05.2021

ABSTRACT

The study was carried to determine the pomological, biochemical and antimicrobial properties of wild blackberry fruit, which we think that is different with its taste and fruit size in the Kelkit valley where is a very rich region in terms of biology diversity due to its different and favorable ecological conditions. At the harvest, 5 kg fruit was collected. Fruit was immediately transported at 10±1.0 °C and 80±5.0 for 2 h by frigorific vehicles to postharvest physiology laboratory of Horticulture Department of Ordu University. Pomological, biochemical and antimicrobial measurements and analyses were made on fruits. Fruit width, length, height and weight were 15.84 mm, 15.37 mm, 15.72 mm and 2.16 g respectively. In the study, the soluble solids content (SSC) ratio, titratable acidity and vitamin C concentration of the genotype was determined as 9.2%, 1.34% and 20.1 mg 100 g^{-1} FW respectively. Total phenolic content was 2158 mg GAE kg^{-1} FW, total flavonoid was 544 mg QE kg^{-1} FW, DPPH and FRAP (antioxidant activity) values were 2749 μmol TE kg^{-1} and 6379 μmol TE kg^{-1} respectively. The solution obtained in blackberry in the study had an antimicrobial effect on all bacteria and fungi. The highest antimicrobial activity against bacteria was obtained against *Escherichia coli*, while the lowest effect was against Klebsiella pneumonial bacteria. In fungi, it was determined that the effect on *Candida albicans* was higher. The study is very important in terms of being a source for future breeding studies in this sense.

Keywords: *Escherichia coli*, bioactive compounds, fruit size, *Rubus fruticosus*.

Citation: Aglar, E., Sumbul, A., Karakaya, O., Erturk, O., and Ozturk, B. 2021. Biochemical Properties and Antimicrobial and Antioxidant activity of Blackberry Growing Naturally in Kelkit Valley. *Journal of Postharvest Technology*, **9**(3): 127-135.

INTRODUCTION

In Anatolia, one of the most important regions of the world in terms of plant population diversity, many wild fruit species naturally survive (Artık and Eksi, 1988). Blackberry, one of these wild fruit species, have been collected and used in human nutrition since ancient times. It is a fruit species that attracts the attention of researchers, producers and consumers with its importance in

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ISSN2348-4330

human health and nutrition due to its delicious taste, pleasant flavour (Zia-Ul-Haq et al., 2014) and presence of basic nutrients, fiber, essential micronutrients, such as minerals, vitamins and various phenolic compounds (Souza et al., 2014). In blackberry, which can easily adapt to different ecological conditions, the fruit quality characteristics and bioactive compounds content, which determines the fruit's potential in terms of health and nutrition, can vary depending on the ecology in which it grows (Grace et al., 2014; Lila, 2006). However, these wild fruit species (Bell, 1995), which are the most important elements of biodiversity, are very valuable with their potential to be an important genetic resource for breeding studies.

Turkey where has different ecological geographic regions is one of the most important countries the fore with its rich genetic resources. However, there are important problems in the detection and evaluation of these genetic resources. Kelkit Valley, which is a geographic transition region where the Central Black Sea and Central Anatolia regions intersect, is a region rich in terms of biodiversity due to its different and favourable ecological conditions. In the Kelkit valley, which includes wild fruit species such as hazelnut, cranberry, plum, apple, hawthorn, raspberry, rosehip, blackberry (Karaer, 1994), there are very limited studies on the unearthing of these genetic resources. In the study planned by considering this situation, it was aimed to determine the pomological biochemical and antimicrobial properties of the fruit belonging to the genotype, which attracts our attention with its fruit size and taste among the wild blackberry genotypes in the region.

MATERIALS AND METHODS

The study was carried out in the Kelkit Valley in 2020. At the harvest, 5 kg fruit in the blackberry growing naturally in Kelkit Valley was collected. Fruit were immediately transported at 10±1.0 °C and 80±5.0 for 2 h by frigorific vehicles to postharvest physiology laboratory of Horticulture Department of Ordu University. Pomological, biochemical and antimicrobial measurements and analyses were made on the fruit.

Fruit size

Fruit width, length and height were determined using a digital calliper (Mitutoyo, Japan) with a precision of 0.01 mm and expressed in mm. The weight of the fruit was measured with a digital scale (Radwag, Poland) with a sensitivity of 0.01 g and expressed as g.

Soluble solids content (SSC) and titratable acidity

The homogeneous fruit juice obtained from 500 g fruit was dropped sufficiently to the digital refractometer (PAL-1, Atago) to measure the SSC, and the value on the screen was recorded as%. For titratable acidity (TA) measurements, 10 ml of fruit juice was taken and 10 ml of purified water was added on it. Then, samples were expressed in terms of malic acid (g malic acid 100 mL^{-1}) based on the amount of NaOH spent in titration with 0.1 N sodium hydroxide until pH 8.1 was reached.

Vitamin C, total phenolics, total flavonoids and antioxidant activity (FRAP and DPPH assays)

0.5 ml of the fruit juice sample was taken, 0.5% oxalic acid was added on it, and it was completed to 5 ml. The ascorbic acid test kit (Catalog No. 116981, Merck, Germany) was dipped to the solution for 2 seconds, it was left outside for 8 sec to be oxide and then the reading was made by inserting it into the test adapter of the reflectoquant device (Merck rqflex plus 10, Turkey) until 15th seconds. The results are expressed as mg 100 g^{-1} (Ozturk et al., 2019).

Total phenolics were determined by using the Folin-Ciocalteu reagent as described in Singleton and Rossi (1965)' study. The fruit extract was left by mixing Folin-Ciocalteu and pure water in a ratio of 1: 1: 20 and then 7% sodium carbonate was added. After two hours of incubation, the solution, which turned into a bluish color, was measured with the spectrophotometer at 750 nm wavelength and the results were calculated as mg gallic acid equivalent (GAE) kg^{-1} fresh weight (FW). Total flavonoids were determined according to the method of Zhishen et al. (1999) and was stated as mg QE (quercetin equivalent) kg^{-1} FW.

DPPH free radical scavenging activity, the hydrogen atom or electron donation abilities of some püre compounds were measured by the bleaching of a purple colored methanol solution of DPPH. The free radical scavenging activities of methanol extract of fresh fruit were measured by 1.1-diphenyl-2-picrylhydrazil (DPPH·) using the method of Blois (1958) wherein the bleaching rate of a stable free radical, DPPH· was monitored at a characteristic wavelength in the presence of the sample. An amount of 0.5 mL of 0.1mM ethanolic solution of DPPH was added to 3.0 mL of all the extract samples or standard antioxidants solution (50–500 μg mL^{-1}) in water. The mixture was shaken vigorously and kept standing at room temperature for 30 min. Then the absorbance of the mixture was measured at 517 nm. The results were expressed as μmol Trolox equivalents (TE) kg^{-1} FW (Demirtas et al., 2013).

For the FRAP (ferric ions (Fe+3) reducing antioxidant power assay), portions of 120 μL were taken from the samples, 0.2M of phosphate buffer (PO4 −3) (pH 6.6) was added to obtain a volume of 1.25 mL and then 1.25 mL of 1% potassium ferricyanide (K3Fe(CN)6) solution was added. After vortexing, they were incubated at 50 °C. Afterwards, 1.25 mL of 10% TCA (trichloro acetic acid) and 0.25 mL of 0.1% FeCl3 were added to the samples. The absorbance of the resultant solution were read on an UV–vis spectrometer at 700 nm. The results were expressed as μmol TE kg^{-1} FW (Benzie and Strain, 1996).

Antimicrobial effect

Preparation of Honey and pollen samples Extracts: Blackberry fruit were pulped by extruding firstly. Then 15 ml pulp was took and it was completed 40 ml with 80% alcohol and centrifuged at 4000 x rpm for 10 minutes. A solution with a concentration of 80 mg was prepared from the supernatant suspension in the upper part and the antimicrobial effect of this solution was examined.

Bacterial strains and growth conditions*:* The antimicrobial activity of Chemistry samples were studied using bacteria *Pseudomonas aeruginosa* ATCC®27853 Gram (-),*Enterococcus feacalis* ATCC® 29121(+)*, Escherichia coli* ATCC®25922 Gram (-),*Klebsiella pneumoniae* ATCC®13883 Gram (-)*, Bacillus subtilis* B209, Gram (+),*Bacillus cereus* ATCC®10876 Gram (+),Mueller Hinton Agar (MHA, Merck) or Mueller Hinton Broth (MHB, Merck) *Candida albicans ATCC®10231 and Aspergillus niger ATCC 9642* Sabouraud Dextrose Broth (SDB, Difco) or Sabouraud Dextrose Agar (SDA, Oxoid) were used for growing bacterial and yeast or fungal cells, respectively

Antibacterial and antifungal assay: Antibacterial and Antifungal activity were measured using methods of disc diffusion on agar plates (Erturk, 2006). In order to test antibacterial and antifungal activity, the fractions of mad honeys and pollen samples were dissolved in ethanol, were investigated by broth microdilution method according to the Clinical and Laboratory Standards Institute standard procedures. All bacterial strains were grown in Mueller Hinton Broth medium (Merck) for 24 h, at 37°C and fungal strains were grown in Sabouraud Dextrose Broth (Difco) at 30°C for 48 h. Bacterial suspension turbidity 0.5 McFarland and fungal suspension turbidity 1.0 McFarland standard were prepared. Thus, the concentration of bacterial suspensions were adjusted to 10^8cells mL^{-1}, and fungal suspension to $3x10^8$cells. Then, sterile paper discs (6 mm in diameter) were placed on the agar to load 25 μl of each orchids plant (80 mg mL^{-1}). One hundred units of nystatin for fungus and streptomisin 10mcg and

Piperasilin 100mcg for bacteria, all obtained from a local pharmacy, were used as positive controls and alcohol as a negative control. Inhibition zones were determined after incubation at 27°C for 48 h. Inhibition zones of different organism by different samples were measured with the help of the digital caliper for the estimation of potency of antibacterial and antifungal substance and tabulated. All tests were made in triplicate.

RESULTS AND DISCUSSION

Fruit size

Fruit size in blackberries is a significant criterion in both fresh consumption and processing, and the fruit are asked to be between 8-10 g. (Clark and Finn 2011). While the fruit size varies depending on the variety, the species and the ecological factors of the region where it is grown, the cultivation status of the species is one of the most significant factors in fruit size. The fruit of the wild blackberry species are smaller than the fruit of cultivated species. The fact that, Yılmaz et al. (2009) reported that fruit weight of blackberry cultivars ranged between 1.2 g (cv. Arapaho) and 5.4 g, with cv. Bursa 1 having the biggest fruits, but the average fruit weight of wild genotypes was from 0.4 g (R3) to 1.2 g (R12). In Turkey, the fruit weight of the wild blackberry species varies between 1.50- 2.11 g (Celik et al., 2003) while the fruit weight of the cultivated species is between 2.00-6.82 g (Cangi and Islam, 2003; Gercekcioglu et al., 2003; Agaoglu et al., 2007). The fruit of the wild genotype that was used in the study were found to be larger when compared with the previous study results (Celik et al., 2003). Fruit width, length, height and weight were 15.84 mm, 15.37 mm, 15.72 mm and 2.16 g respectively (Table 1). Ecological factors of the region and genotype may have been effective in the emergence of this result. With this feature, this genotype can be evaluated in future breeding studies.

Table 1: The fruit size and biochemical properties of the blackberry Kelkit Valley

Fruit weight (g)	2.16	Soluble solids content (%)	9.2	Total flavonoids (mg QE kg^{-1})	544
Fruit length (mm)	15.37	Titratable acidity (%)	1.34	Antioxidant activity (μmol TE kg^{-1})	
Fruit width (mm)	15.84	Vitamin C (mg $100g^{-1}$)	20.1	FRAP	6379
Fruit height (mm)	15.72	Total phenolic (mg GAE kg^{-1})	2158	DPPH	2749

Soluble solids content and titratable acidity

The chemical composition of the blackberries varies depending on the species, variety, growing conditions, harvest period and time. The amount of SSC (Abiodun and Akinoso, 2014), which is one of the ripening criteria in fruit and an important parameter affecting consumer preferences in the food industry (Abiodun and Akinoso, 2014), varies between 4-12% in blackberries. The cultivation status of the species may cause differences in the SSC rate, and the SSC rate in wild species is higher than in cultivated varieties (Yılmaz et al., 2009). In the study, the SSC rate of the genotype was determined as 9.2%. When compared with similar studies, it can be said that the SSC ratio of the genotype is relatively high (Table 1). In fact, in a study conducted to determine quality characteristics in 10 blackberry genotypes in Colombia (Sánchez-Betancourt et al., 2020), it was reported that there were significant differences between genotypes in terms of SSC content and the SSC ratio varied between 5.68 -7.55. Again, Eskimez et al. (2019) suggested that the SSC rate in blackberries was between 7.1-9.5% while Moraes et al. (2020) who has reported that the SSC ratio occurs the differences depending on the cultivars, has recorded that the SSC ratios in Guarani, Tupy, Xavante and BRS Xinqu cultivars were 7.5, 7.3, 9.0 and 9.0 respectively. Faedi and Rosati (1978), who obtained results consistent with our study, determined that the SSC rates of Black Satin and Dirksen Thornless cultivars were 8% and 9.46% respectively. Blackberry cultivars grown in different regions of Turkey had soluble solids (8.98%–20.2%), and acidity (1.0%–

3.1%) for cultivated blackberry while TSS (11.3%–13.1%), and acidity (0.7%–1.0%) for wild blackberries (Agaoglu et al., 2007; Gercekcioglu et al., 2003).

The type and amount of acid found in blackberries may vary depending on the species, cultivar, fruit maturity and ecology (Tosun and Artık, 1998). Plowman (1991), who stated that the dominant acid in blackberry changes depending upon cultivar and species, suggested that citric acid is more dominant in Black satin, Boysenberry and Loganberry cultivars, and isocitric and malic acid in Georgia Thornless and Smootshem cultivar. Nizharadze et al. (1977) reported that titratable acidity in blackberry was 1.47% as malic acid while Patschky and Schoene (1972) reported that the ratio of malic and citric acid was 1.00 %. Yılmaz et al. (2009), who stated that there is no significant difference in terms of the titration acidity between wild and cultivated species, suggested that the titratable acidity in blackberries was between 0.5 and 1.5 while Moraes et al. (2020) recorded that the titratable acidity was between 0.76 and 1.04 in their study of four different cultivars. Considering these explanations, it can be said that the 1.34 titration acidity value determined in our study is relatively high.

Vitamin C, phenolics, total flavonoids and antioxidant activity (FRAP and DPPH)

In the study, the vitamin C concentration of the wild blackberry fruit was determined as 20.1 mg 100 g^{-1} FW (Table 1). When compared with similar studies, it was seen that the genotype, which attracts our attention with its fruit size and taste among the wild blackberry genotypes in the Kelkit Valley, was quite rich in vitamin C. Indeed, Yildiz et al. (2010), who determines the quality and chemical composition parameters of the fruit of 10 wild blackberry genotypes in the Coruh Valley, reported that the vitamin C contents in these genotypes were between 14.05 and 17.15 mg 100 g^{-1} FW. Again, Akin et al. (2016) reported that in their study conducted in the Black Sea region, the vitamin C ratio in the wild blackberries was 13.33 mg 100 g^{-1} fw. Ochmian et al. (2009) determined that the vitamin C ratio of the wild blackberries in Poland (Szczecin) was as 11 mg 100 g^{-1}.

Blackberries are rich in phenolic compounds such as anthocyanins, flavonols, chlorogenic acid and procyanidins, which have high biological activity and can provide health benefits as dietary antioxidants (Cho et al., 2005; Koca and Karadeniz, 2009; Zia-Ul-Haq et al., 2014). The content and concentration of the bioactive compounds in blackberries varies depending on the species, cultivar, ecological conditions and cultural practices (Scalzo et al., 2005), while the wild blackberries are richer in terms of the total phenolic content (Reyes-Carmano et al., 2005; Yılmaz et al., 2009; Mikulic-Petkovsek et al. 2012) and antioxidants (Yildiz et al., 2010) than cultivated cultivars. Yilmaz et al. (2009) have suggested that an increase in the polyphenolics concentration of the wild fruit species may be occurred by stimulating the synthesis of antioxidant enzymes as a defence mechanism in these species as the wild fruit species are more exposed to extreme temperatures and are vulnerable to diseases and pests. Yildiz et al. (2010) reported that the average total phenolic contents of wild genotypes were 381 mg GAE 100 g^{-1} FW indicating higher value than cv. Chester (310 mg 100 g^{-1} FW). In a study conducted to determine the physicochemical properties of the wild and cultivated cultivars in blackberries (Yılmaz et al., 2009), it was reported that there were the significant differences between the cultivars and wild genotypes in terms of physicochemical properties and the total phenolic content of the cultivated cultivars was between 584 -788 mg 100 g^{-1} FW while in wild genotypes was between 610 -1455 mg 100 g^{-1} FW. In the same study, it has been determined that the antioxidant activity of the wild genotypes was lower and the average antioxidant activities of the wild genotypes and cultivated cultivars were 76.2% and 81.9% respectively.

In our study, it was determined that total phenolic content, total flavonoid, DPPH and FRAP (antioxidant activity) values were 2158 mg GAE kg^{-1} FW, 544 mg QE kg^{-1} FW, 2749 μmol TE kg^{-1} and 6379 μmol TE kg^{-1}, respectively (Table 1). Mertoglu et al. (2021), who has reported that the changes in the concentration of bioactive compounds occur depending on the cultivar, in his study, it was reported that the total phenolics, antioxidant activity, total monomeric anthocyanin and total flavonoids were between 718.3-1042.5 mg GAE 100 mL^{-1}, 47.6-63.7%, 30.1-60.3 mg cyndn-3-glucoside 100 mL^{-1} and 29.1-44.1 mg catechin 100 mL^{-1} respectively.

Antimicrobial and antifungal effect

Antimicrobial activity in the sample obtained by cold press technique in the fruit of the genotype, which attracts our attention with its fruit size and taste in the wild blackberry genotypes in the Kelkit Valley, which is a very rich region in terms of biology diversity due to its different and favourable ecological conditions, was tested on two fungi (*Candida albicans* and *Aspergillus niger*) and six bacteria including three Gram positive (*Enterococcus feacalis, Bacillus subtilis* and *Bacillus cereus*) and three Gram negative (*Pseudomonas aeruginosa, Escherichia coli* and *Klebsiella pneumonial*) according to disc diffusion on agar plates.

Table 2: The antimicrobial properties of the blackberry in Kelkit Valley

SAMPLES	*B. subtilis*	*B. cereus*	*K. pneumoniae*	*E. coli*
Rubus fruticosus	15.89±0.46	16.36±0.17	11.74±0.50	19.11±0.41
Piperasilin 100mcg	6.0±0.00	12.23±0.29	6.0±0,00	6.0±0,00
Streptomisin 10mcg	14.63±0.00	6.0±0,00	18.16±0.30	21.34±0.17
Nystatin	NT	NT	NT	NT
Solvent	6.0±0.00	6.0±0,00	6.0±0,00	6.0±0,00
SAMPLES	***A. niger***	***E. faecalis***	***C. albicans***	***P. aeruginosa***
Rubus fruticosus	12.96±0.76	16.29±0.53	16.15±0.28	14.52±0.54
Piperasilin 100mcg	NT	6.0±0,00	NT	17.11±0.57
Streptomisin 10mcg	NT	21.03±0.44	NT	6.0±0,00
Nystatin	16.76±0.55	NT	17.3±0.32	NT
Solvent	6.0±0,00e	6.0±0,00d	6.0±0,00	6.0±0,00

no inhibition, NT: Not tested, *Pseudomonas aeruginosa* ATCC®27853 Gram (-), *Enterococcus faecalis* ATCC® 29121(+), *Escherichia coli* ATCC®25922 Gram (-), *Klebsiella pneumoniae* ATCC®13883 Gram (-), *Bacillus subtilis* B209, Gram (+), *Bacillus cereus* ATCC®10876 Gram (+),*Candida albicans ATCC®10231, Aspergillus niger ATCC 9642*

The solution obtained in blackberry in the study had an antimicrobial effect on all bacteria and fungi. The highest antimicrobial activity against bacteria was achieved against *Escherichia coli* while the lowest effect was against *Klebsiella pneumoniae* bacteria. In fungi, it was found that the effect on *Candida albicans* was greater (Tables 2). In the study that was conducted to determine the antimicrobial effect of four blackberry cultivars it had been used *Escherichia coli, Enterococcus faecalis* and *Staphylococcus aureus* bacteria and *Candida albicans* and *Candida parapicilosis* fungi as strains and it had been reported that the anti-microbial effect in blackberries varied between cultivars (Mertoglu et al., 2021).

As a results, the wild blackberry genotypes in the Kelkit Valley, which is a very rich region in terms of biology diversity due to its different and favourable ecological conditions has the potential to be a valuable genetic resource with its superior properties such as taste, fruit size and phytochemical content. This study is very valuable in terms of revealing the existence of this genetic potential and being a guide for the improvement studies to be made in this sense, and it should be shared with the addressees.

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