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ORIGINAL ARTICLE / ORIGINALBEITRAG

Phytochemical and Antimicrobial Characteristics of Raspberry Fruit Growing Naturally in Kelkit Valley, Turkey

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Abstract
The study was carried out to determine the pomological, phytochemical and antimicrobial properties of wild raspberry fruit *(Rubus idaeus)* naturally growing in the Kelkit Valley in Turkey. In the study, the wild raspberry fruit was comparatively smaller. In fruit, soluble solids content (SSC) was determined as 12.6%, titratable acidity as 1.36% and vitamin C concentration as 28.8 mg 100 g^{-1}. Total phenolics, total flavonoids, 1,1-diphenyl-2-picrylhydrazil (DPPH) and ferric ions (Fe+3) reducing antioxidant power assay (FRAP) activity values were 1775 mg kg^{-1}, 151 mg kg^{-1}, 2580 µmol kg^{-1} and 5187 µmol kg^{-1}, respectively. In the study, the solution obtained from raspberries had an antimicrobial effect on bacteria. While it had an antimicrobial effect on *Aspergillus niger* fungus, it did not have any effect on *Candida albicans* fungus. The highest antimicrobial activity was achieved against *Pseudomonas aeruginosa*, while the lowest effect was against *Enterococcus feacalis* bacteria. It was revealed that the raspberry fruit investigated in the study can be used as material for breeding studies due to their rich bioactive compounds and antimicrobial content.

Keywords Flavonoids · Phenolics · *Pseudomonas aeruginosa* · *Rubus idaeus* · Vitamin C

Introduction

The wild fruit species that adapt to the ecological conditions of the regions they grow in are more resistant to diseases and pests (Karagoz et al. 2010) and are very important in the development of varieties that are resistant to diseases and pests, as well as suitable for consumer preferences and different ecological conditions (Agaoğlu et al. 1995). However, the positive impact of wild species on human nutrition and health increases interest in these species. Anatolia, which is located in the Near East and Mediterranean plant gene centres and hosts many wild fruit species, is a very rich region in terms of gene resources. Having said that, it is apparent that the efforts to utilize and evaluate these gene resources are not sufficient.

The cultivation of many fruit species indigenous to Turkey has only recently begun. There are also species, the presence of which we are not aware of. The best example of this is raspberry. The raspberry, which has anti-obesity, anti-neurodegenerative and anti-cancer properties because it is rich in phenological compounds and antioxidants such as anthocyanins, tannic acid and flavonoids, has long been used in human health and nutrition (Zafrilla et al. 2001; Coates et al. 2007; Fu et al. 2015). However, the raspberry is a fruit species that has only recently been cultivated in Turkey. Nevertheless, the wild raspberry species that grow naturally at an altitude higher than 1000 m in the Marmara and Black Sea regions (Onur et al. 1999) have been collected and consumed for a long time. The increasing importance of the wild fruit species in human health, nutrition and breeding studies has led to an increase in interest in these species. Raspberry, which draws attention with its unique smell, taste, flavor, aroma and positive effect on human health with its phenols, flavonoids and vitamins (Kahkönen et al. 1999; Halvorsen et al. 2001; Okatan 2020), is one of the most significant of these wild species.

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The Kelkit Valley, which is an ecotone region rich in wild fruit species potential, is one of the areas of natural spread of raspberry. However, the potential of this wild raspberry, which has the ability to grow in very high and restricted areas, is not well known by the local people and scientists. The potential of these wild raspberry forms to be a genetic source, as well as the possibility that the chemical content in the fruit may differ due to both the altitude where it grows and its location in a limited area, made it necessary to carry out this study. The main aim of this study, which is preliminary research to reveal this genetic source, is to determine the pomological biochemical and antimicrobial properties of the wild raspberry fruit.

Materials and Methods

Plant Material

The study was carried out in the Kelkit Valley, Turkey, in 2018. At harvest time (10 August), a total of 5 kg fruit of the raspberry growing naturally in the Kelkit valley was collected for pomological and biochemical measurements and analyses. The fruit was immediately transported at 10 ± 1.0 °C and 80 ± 5.0 humidity for 2 h by frigorific vehicles to the postharvest physiology laboratory at the Horticulture Department of Ordu University. Pomological, biochemical and antimicrobial measurements and analyses of the fruit were performed.

Fruit Size

Fruit size was determined by measuring the width, height and weight of 20 fruit. Fruit width, length and height were determined by means of digital calipers (Mitutoyo, Japan) with 0.01-mm precision and expressed in millimeters. The weight of each fruit was measured using a digital scale (Radwag, Poland) with a sensitivity of 0.01 g, and the fruit weight was determined by taking averages expressed in grams.

Soluble Solids Content and Titratable Acidity

In all, 500 g of the fruit was crushed with a blender and homogenized. The homogenate obtained was passed through a cheesecloth and juice was obtained. A sufficient amount of juice was dropped into the digital refractometer (PAL-1, Atago) for SSC measurement, and the value on the screen was recorded in percent. For titratable acidity (TA) measurements, 10 ml of the juice was taken and 10 ml of distilled water added to this. The samples were then expressed in terms of malic acid (g malic acid 100 ml^{-1}) based on the amount of sodium hydroxide (NaOH) spent in titration with 0.1 N NaOH until pH 8.1 was reached (Ozturk et al. 2019a).

Vitamini C, Total Phenolics, Total Flavonoids and Antioxidant Activity

For vitamin C, the homogenate was filtered through a cheesecloth, and 0.5 mL juice was obtained. Then, 5 mL of 0.5% oxalic acid was added to this. The ascorbic acid test strip (Catalog no: 116981, Merck, Germany) was taken from a collapsible sealed gastight tube. The Reflectometer (Merck RQflex plus 10) was started. The test strip was plunged into the solution for 2 s, then removed from the solution. It was then held for 8 s, and the reading was taken at the end of the 15th second. The results were stated as mg 100 g^{-1} (Ozturk et al. 2019a).

A total of 50 fruits were washed with distilled water and sliced with a stainless steel knife. The fruit pulp was later crushed in a blender and homogenized. About 30 mL of the homogenate was obtained and placed in a 50-ml falcon tube. The tubes were kept at –20 °C until analyses could be performed. Before beginning analyses, the frozen samples were dissolved at room temperature (21 °C). Pulp and juice were separated from each other by a centrifuge at $12.000 \times g$ at 4 °C for 35 min. The resulting filtrate was used to determine the content of total phenolics, total flavonoids and antioxidant activity. Total phenolics were determined using the Folin-Ciocalteu reagent as described in the study by Ozturk et al. (2019b) and expressed as mg kg^{-1} GAE (gallic acid equivalent) fresh weight (fw). Total flavonoids were determined as described in the study by Ozturk et al. (2017) and was stated as mg kg^{-1} QE (quercetin equivalent) fw.

1,1-Diphenyl-2-picrylhydrazil (DPPH) free radical scavenging activity, the hydrogen atom or electron donation abilities of some pure compounds, were measured by bleaching a purple colored methanol solution of DPPH. The free radical scavenging activities of methanol extract of fresh fruit were measured by 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 0.1-mM ethanolic solution of DPPH was added to 3.0 mL of all the extract samples or standard antioxidant solution (50–500 µg mL^{-1}) in water. The mixture was shaken vigorously and kept standing at room temperature for 30 min. The absorbance of the mixture was then measured at 517 nm. The results were expressed as µmol trolox equivalents (TE) kg^{-1} fw (Ozturk et al. 2019c).

For the ferric ions (Fe^{+3}) reducing antioxidant power assay (FRAP), portions of 120 µL were taken from the samples, 0.2 M 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, these were incubated at 50 °C. Afterwards, 1.25 mL of 10% trichloro acetic acid (TCA) and 0.25 mL of 0.1% $FeCl_3$ were added to the samples. The absorbances of the resultant solution were read on a UV-vis spectrometer at 700 nm. The results were expressed as µmol TE kg^{-1} fw (Ozturk et al. 2019b).

Antimicrobial and Antifungal Effect

Raspberry fruit were pulped firstly by extrusion. Then, 15 ml of pulp was taken, completed to 40 ml with 80% alcohol and centrifuged at 4000 × g for 10 min. 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 the 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 and investigated by the 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) for 48 h at 30 °C. Bacterial suspension turbidity 0.5 McFarland and fungal suspension turbidity 1.0 McFarland standard were prepared. Thus, the concentration of bacterial suspensions was adjusted to 10^8 cells/mL and fungal suspension to 3×10^8 cells. Sterile paper discs (6 mm in diameter) were then placed on the agar to load 25 µl of each orchard plant (80 mg/mL). A total of 100 units of nystatin for fungus and streptomycin 10 mcg and piperacillin 100 mcg 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 for 48 h at 27 °C. 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 were presented as mean ± standard deviation.

Results and Discussion

Fruit size

In raspberry, fruit size varies depending on fruit species and environmental factors. The fruit of the wild raspberry species are smaller than those of the cultivated species. In wild species, the fruit weight ranges from 1.1 to 1.6 g (Petrovic and Milosevic 2002), while the fruit weight in cultivated varieties is between 3 and 6 g (Misic and Nikolic 2003). These differences may be due to genetic differences between wild and cultivated varieties as well as cultural practices in cultivated varieties. The fruit of the wild raspberry, which grows naturally on the northern slope of a mountain with an altitude of 2500–2800 m in the Kelkit valley, was observed to be relatively smaller (Table 1). The species growing in the region, the altitude of the region and ecological factors may have contributed to the occurrence of smaller fruit. Fruit quality properties of these wild fruit species, which adapt to the ecological conditions of the region where they grow and have developed a resistance mechanism against biotic and abiotic environmental factors, may be affected by these factors (Wang and Lin 2000). Indeed, Kulina et al. (2012) reported that the color and size of the wild raspberry fruit can vary depending on the species and environmental factors. In their study, the fruit weight, length, width and height values were recorded as 1.01 g, 11.97 mm, 12.45 mm and 13.13 mm, respectively (Table 1). It is evident that the wild raspberry fruit is very small compared to the cultivated

Table 1 Fruit size and biochemical properties of the raspberry in Kelkit Valley

Fruit quality characteristics	Mean ± standard deviation
Fruit weight (g)	1.01 ± 0.23
Fruit length (mm)	11.97 ± 1.13
Fruit width (mm)	12.45 ± 1.32
Fruit height (mm)	13.13 ± 0.75
Soluble solids content (%)	12.6 ± 1.06
Titratable acidity (g malic acid 100 ml^{-1})	1.36 ± 0.26
Vitamin C (mg 100 g^{-1})	28.8 ± 2.16
Total phenolics (mg GAE kg^{-1})	1775 ± 12.38
Total flavoniods (mg QE kg^{-1})	151 ± 7.16
FRAP (µmol TE kg^{-1} fw)	5187 ± 24.3
DPPH (µmol TE kg^{-1} fw)	2580 ± 15.1

FRAP Ferric ions (Fe+3) reducing antioxidant power assay, *DPPH* 1,1-Diphenyl-2-picrylhydrazil cultivars. In previous studies, it was determined that fruit weight in the 'Willamette' (Stanisavljevic et al. 2004), the 'Meeker' and the 'Heritage' cultivars were 4.72 g, 4.00 g and 3.49 g, respectively (Kulina et al. 2012).

Soluble Solids Content and Titratable Acidity

The study determined that the SSC rate in raspberry fruit was 12.6% and titratable acidity was 1.36% (Table 1). Supporting our study results, Eke (2017) reported that the SSC and acidity rate in wild raspberry were 13% and 1.93%, respectively, while Purgar et al. (2012) on the other hand, found that titratable acidity and SSC in *Rubus idaeus* species in Croatia was 1.74% and 10.5%, respectively. Soskic (1989) reported that the total acidity content of the wild raspberry species was between 1.57 and 1.91% and the SSC was between 10 and 12%, while Fotirić et al. (2009) recorded a total acidity content of between 0.55 and 1.14% in wild raspberry species. There are significant differences in raspberry between cultured and wild species according to region in terms of SSC and acidity rates. In fact, it was determined that SSC in the wild raspberry species was 9.4–11.5% in Croatia (Purgar et al. 2012), 7.1–10.8% in Greece (Pantelidis et al. 2007), 7% in Iran (Nalbandi et al. 2011) and 9.95–12.80% in Serbia (Fotirić et al. 2009), while SSC rate in our study was 12.6% (Table 1). The genetic characteristics of the variety and the ecological factors of the region may contribute to the differences occurring in SSC and acidity rates in raspberry according to species, variety and region. Indeed, Milosević (1997) reported that the content of basic chemical compounds varies depending on raspberry type, ecological conditions and level of practical measures applied.

Vitamin C, Total Phenolics, Total Flavonoids and Antioxidant Activity

Raspberry and blackberry, with their high levels of anthocyanins, flavonoids and phenolic acids (Wang and Lin 2000), are a good source of natural antioxidant substances, which may exhibit a wide range of biological effects, including antioxidant, antimicrobial, anti-inflammatory and vasodilatory actions (Kahkonen et al. 2001). Due to these properties, these fruit species have long been collected and consumed worldwide (Lee et al. 2012). Wang and Lin (2000) and Nalbandi et al. (2011) revealed the presence of bioactive compounds such as phenol, anthocyanin and ascorbic acid in these fruit species, while the content and concentration of these compounds differ greatly between species (Pantelidis et al. 2007). However, the concentration of phenolic compounds in the wild berry species is higher than those in cultivated species. The wild fruit species are more exposed to extreme temperatures and are defenseless to disease and pests. For this reason, antioxidant enzyme synthesis is stimulated as a defense mechanism in these species, and thereby the concentration of polyphenolics increases (Yilmaz et al. 2009).

In the present study, vitamin C concentration in the wild raspberry fruit was determined as 28.8 mg 100 g^{-1} fw (Table 1). Compared to the results of other studies, it can be said that this wild raspberry fruit, which had the ability to grow in a limited area in the region, is rich in vitamin C. According to the 2019 report of the United States Department of Agriculture (USDA), 100 g of raspberry fruit contains 26.2 mg of vitamin C, while Purgar et al. (2012), who reported that the ascorbic acid content is more variable in raspberry species, determined that the ascorbic acid content in *R. idaeus* genotypes ranged from 22.34 to 45.00 mg 100 g^{-1}. Again, Milivojević et al. (2010) stated that the ascorbic acid content in raspberry varieties cultivated in Greece was 40.9 mg 100 g^{-1} fw (Willamette) and 44.3 mg 100 g^{-1} fw (Meeker), while the wild raspberry fruit in Serbia was richer in ascorbic acid content (56.1 mg 100 g^{-1} fw).

In the present study, total phenolics, total flavonoids, DPPH and FRAP (antioxidant activity) were determined as 1775 mg kg^{-1} fw, 151 mg kg^{-1} fw, 2580 µmol kg^{-1} and 5187 µmol kg^{-1}, respectively (Table 1). Compared to similar studies, it can be said that raspberries grown in the Kelkit Valley are relatively rich in bioactive compound content. Indeed, in their study, Purgar et al. (2012) reported on the bioactive compound content of the wild raspberry in Croatia: total phenolics was 345 and 483 mg kg^{-1}, the amount of anthocyanin was 279–582 mg kg^{-1} and non-flavonoid content was 204 and 246 mg kg^{-1}. Milivojević et al. (2010) determined the total phenolics in raspberry to be 1020–2220 mg kg^{-1} in the cultivated varieties and 1100 mg kg^{-1} in the wild species. In Greece, Pantelidis et al. (2007) determined the phenolics of raspberry cultivars, which varied from 657 to 2494 mg GAE kg^{-1}.

Antimicrobial and Antifungal Effect

With the microorganisms becoming resistant to artificial antibiotics, the importance of wild plants, which are a source of natural compounds used in the treatment of various diseases and ailments and which act as antimicrobial drugs and anti-infection agents, is increasing day by day (Rios and Recio 2005). These plants, which can adapt and grow under different climatic conditions, have a positive effect on human health with their high antioxidant capacity and antimicrobial properties. Antimicrobial activity in the sample obtained by cold press technique in the wild raspberry fruit, which has adapted to extreme climatic conditions at an altitude of 2500–2800 m in the Kelkit valley, was tested on six bacteria (three Gram-negative and three Gram-positive)

Table 2 Antimicrobial properties of the raspberry in Kelkit Valley

Samples	*B. subtillis*	*B. cereus*	*K. pneumoniae*	*E. coli*
Rubus idaeus	10.25 ± 0.53	11.36 ± 0.71	10.79 ± 0.52	11.97 ± 0.66
Piperacilin 100 mcg	6.0 ± 0.00	12.23 ± 0.29	6.0 ± 0.00	6.0 ± 0.00
Streptomycin 10 mcg	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. fecalis*	*C. albicans*	*P. aeruginosa*
Rubus idaeus	11.27 ± 1.20	9.43 ± 0.79	0.9 ± 0.36	12.07 ± 0.087
Piperacilin 100 mcg	NT	6.0 ± 0.00	NT	17.11 ± 0.57
Streptomycin 10 mcg	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, *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 (+), *Candida albicans* ATCC®10231, *Aspergillus niger* ATCC 9642
NT Not tested

and two fungi according to disc diffusion on agar plates. The solution obtained from raspberry fruit in the study had an antimicrobial effect on bacteria: It had an antimicrobial effect on *A niger* fungus, while it did not have any effect on *C. albicans* fungus. The highest antimicrobial activity was achieved against *P. aeruginosa*, while the lowest effect was against *E. feacalis* bacteria (Table 2). However, Demirkol and Erturk (2019), in their study to determine the antimicrobial effect in rosehip and sumac, three Gram-positive (*B. cereus, Staphylococcus aureus* and *Listeria monocytogenes)* and four Gram-negative (*Proteus vulgaris, E. coli, Salmonella typhimurium* and *Pseudomanas aeruginosa*) were used as strains, and unlike our study, they reported that the most resistant bacteria was *P. aeruginosa*, that is, the lowest antibacterial effect was seen in this bacteria. Also in that particular study, the Gram-positive bacteria were more sensitive than Gram-negative bacteria. However, in our study, there were no differences in antimicrobial effect due to the fact that the bacteria used as strains were Gram-negative or Gram-positive, except for *E. feacalis* (Table 2). In support of the study results, Sabatini et al. (2020) also reported that there were no significant differences in antibacterial activity between Gram-negative and Gram-positive bacteria in *Prunus spinosa*. However, Erturk et al. (2017) reported in their study on 48 different fruit and vegetable species that the extracts obtained were more effective against Gram-negative bacteria than against Gram-positive ones.

However, it is known that Gram-positive bacteria are more sensitive to plant extracts than Gram-negative bacteria (Cosentino et al. 1999; Karaman et al. 2001). Ngwoke et al. (2011) reported that plants generally have a much greater inhibition effect against Gram-positive than Gram-negative bacteria. The differences in the antimicrobial activity of plant extracts against Gram-positive and Gram-negative bacteria are due to the different cell structure of these bacteria (Hasheminya et al. 2018). Hasheminya and Dehghannya (2020) suggested that these bacteria are less susceptible to the antimicrobial effects of plants due to the presence of the outer membrane around the cell wall in Gram-negative bacteria. Gram-positive bacteria do not have this cell membrane and cell wall structure, meaning that antibacterial materials can rapidly destroy the bacterial cell wall and cytoplasmic membrane, which leads to the flow and coagulation of cytoplasm (Cos et al. 2006; Rasooli et al. 2006).

Conclusion

This study is of considerable value and should be shared with the relevant readership since it has revealed the existence of wild raspberries that are capable of growing in a limited area by adapting to the extreme ecological conditions of the region at an altitude of 2500–2800 m in the Kelkit valley, Turkey. The study also has the potential to serve as a guide for future breeding studies.

Conflict of interest E. Aglar, A. Sumbul, O. Karakaya, O. Erturk and B. Ozturk declare that they have no competing interests.

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