

DETERMINATION OF ANTI-MICROBIAL AND PHYTO-CHEMICAL CHARACTERISTICS OF SOME BLACKBERRY CULTIVARS

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ABSTRACT

This study was carried out to determine the phytochemical and anti-microbial properties of some blackberry cultivars namely Chester, Jumbo, Bursa 1 and Bursa 2. According to results the, phenolic compounds such as total flavonoid and anthocyanin content were found in the range of 29.05 (Bursa 2) – (Jumbo) 44.13 mg catechin 100 mL⁻¹ and 30.08 (Bursa 2) - 60.27 (Chester) mg cyanidin-3-glucoside 100 mL⁻¹, respectively. Bursa 1 has been noted prominent in terms of antioxidant activity (63.73%), followed by Bursa 2 (52.84%), Chester (52.5%) and Jumbo (47.57%). Among the blackberry cultivars, Jumbo and Chester were found highly effective against to *Candida albicans* while similar anti-fungal effectiveness was found in Bursa 2 to *Candida parapsilosis*. Moreover, Bursa 2 and Chester showed strong anti-bacterial effects to *Enterococcus faecalis* whereas against to *Staphylococcus aureus* Bursa 1 and Jumbo were determined highly effective. Although antioxidant activity had a high level of positive relationship with total phenol (0.88***), it was negatively correlated with vitamin C (-0.51*) and total flavonoid amount (-0.58**). Due to Anthocyanins are a kind of flavonoid compounds, high positive correlation (0.70***) detected between these two characteristics.

KEYWORDS:

Anti-bacterial, anti-fungal, antioxidant activity, phenolic compounds, *Rubus fruticosus* L

INTRODUCTION

Horticultural crops are very diverse group including numerous cultivars, accessions, genotypes, etc. and many of them are a key source of qualitative nutritive traits and high medicinal properties [1, 2, 3, 4, 5, 6]. So, they have been evaluated in medical besides their fresh consumption for

centuries [7, 8, 9]. One of them, blackberry, has high adaptability to different climatic conditions, contain rich biochemical content and is an important early and regular fruiting species as a complementary product in agribusinesses, especially in terms of assessment of the woman and child labor force, since it requires hand-intensive agricultural force [10, 11].

In today's World, consumer awareness is increasing and the health effects of fruits are expected to be superior in addition to the sensory properties of the harvested crops. With affluent and diverse phytochemical content in addition to its flavor, blackberry is highly valuable in terms of these properties [12, 13].

Phenolic and organic acids are commonly found in blackberry fruits and show high antioxidant activity by preventing oxidation and peroxidation reactions. It has been stated that this antioxidative effect reduces the risk of many chronic diseases including cancer and cardiovascular diseases [14, 15, 16]. So, addition of these compounds in the daily diet is very important in terms of human health, adequate and balanced nutrition.

In recent years, the demand for natural products has been increasing due to the chemicals found in drugs. Resistance of micro-organisms to anti-microbial drugs has led people to plants or plants' by products, which they see as alternative medicine. In this context, the effectiveness of many species has been investigated and studies are continuing extensively throughout world [17, 18, 19, 20].

In this study, some biochemical properties of four different cultivars of blackberry were investigated. In addition, anti-microbial effects of these cultivars against 2 yeast and 3 bacterial strains have also been determined.

TABLE 1
Ecological data of the research area in related years and months

Climatic parameters									
	Temperature (°C)			Precipitation (mm)			Humidity (%)		
	Long term	2017	2018	Long term	2017	2018	Long term	2017	2018
March	5.9	7.3	9.2	59.1	74.4	69.3	66.0	64.1	65.9
April	10.7	10.6	14.2	52.9	25.6	6.3	61.5	59.6	51.0
May	15.4	14.9	16.8	56.7	149.5	62.9	59.2	63.7	62.3
June	19.8	20.1	20.0	33.6	30.9	69.4	52.5	58.9	62.4
July	23.4	25.2	24.3	16.3	13.1	4.1	45.7	41.9	46.9
August	23.2	23.8	24.3	14.3	20.4	14.2	46.4	52.1	47.6
September	18.8	21.0	20.7	18.8	5.7	1.6	52.3	45.1	47.6

Physical and chemical properties of the soil									
pH (1/2.5 water)	Structure	Lime (%)	Organic material (%)	Nutrients that can be extracted (mg/kg)					
				P	K	Fe	Cu	Zn	Mn
8.1	Clay-loam	26.0	1.9	12.6	124	3.1	0.9	0.38	2.9

MATERIALS AND METHODS

Present study was carried out using four different blackberry cultivars (Jumbo, Chester, Bursa 1 and Bursa 2) from 2017 to 2018. Experimental plantations of these four cultivars were located in the fields of Isparta Applied Sciences University, Faculty of Agriculture. The planting was carried out in 2015 by transferring the plants to the field with a row and intra-row spacing of 3.5 x 1.2 m. Plants were irrigated with drip irrigation method and all cultural practices were routinely performed in both trial years.

The altitude of the study area was 1009 m and located at the 37° 50' 13.6464" - 30° 32' 17.6316" coordinates. The ecological data of the research area are given in Table 1. The soil structure of the experimental site is clay-loam with moderate alkaline and the lime content is very high. In addition to organic matter, the amount of extractable P and Zn content were found to be low. K content was medium, Mn, Fe and Cu content were found to be sufficient according to Jackson [21]. For climatic data, it has been observed that the measured average temperature values for months are generally higher than the long term due to the effects of global warming. Regarding the amount of precipitation, when the data of long term is examined, it was observed that there is consistency between the months of the seasons, while irregular precipitation and dry periods were observed in the years covering the study. There is an increasing tendency in humidity compared to long terms, especially in the summer.

Harvest of fruit and preparation for analysis. In order to carry out chemical and anti-microbial analysis, the fruits were harvested in July according to taste, coloration, formation of the abscission layer between the fruit and receptacle and transferred to the laboratory without losing time in both years [11]. Fruits were squeezed to obtain juice with a solid juicer and filtered with coarse filter paper. Obtained

juices were kept at - 20 °C until the analyses were carried out.

Phytochemical analysis. Folin–Ciocalteu method was used for the determination of total phenolic content as defined by Singleton and Rossi [22]. The fruit juices were mixed with Folin–Ciocalteu reagent and distilled water at a ratio of 1:1:18 and left to rest for 8 min, then 7% sodium carbonate was added. After 2 h of incubation in a dark room, the absorbance of the bluish solution was measured at 750 nm. Gallic acid was used as an external standard for the calibration curve and the results were expressed as gallic acid equivalents of fruit juice (mg GAE L⁻¹).

Total flavonoid content of the samples was determined by aluminum chloride colorimetric method according to Chang et al. [23]. 50 µl of juice, 950 µl of methanol and 6400 µl of deionized water were transferred to 10 mL tubes, then 300 µl of sodium nitrite (5% NaNO₂) solution was added. Then 300 µl of aluminum chloride (10% AlCl₃) solution was added to the mixture and it was left for 5 minutes, then 2000 µl sodium hydroxide (4% NaOH) solution was added to the mixture. The mixture was allowed to stand in the dark for 15 minutes and its absorbance was measured at 510 nm using a UV spectrophotometer. Catechin was used as standard in this analysis, and the total flavonoid content was expressed as mg catechin equivalent per mg CAE L⁻¹.

Antioxidant activity analyses were performed using DPPH method. Firstly, 50% inhibition concentration (IC50) was calculated by drawing percent inhibition against the sample concentrations. Then, up to IC 50 value samples were taken and ability to remove the DPPH radical was determined according to the method specified by Polat et al. [24]. Results were expressed as a percentage (%).

Total monomeric anthocyanin content was determined using the pH differential method as described by Selçuk and Erkan [25].

TABLE 2
Phytochemical characteristics of investigated blackberry cultivars

	TPC mg 100 mL ⁻¹	AA %	TMA mg cyndn-3-glukozit 100 mL ⁻¹	TFC mg catechin 100 mL ⁻¹	Vitamin C mg 100 mL ⁻¹
Bursa 1	1042.5 A	63.73 A	40.63 C	30.13 C	18.20 C
Bursa 2	722.5 B	52.84 B	30.08 D	29.05 C	53.04 A
Jumbo	718.3 B	47.57 C	50.13 B	44.13 A	38.97 B
Chester	749.9 B	52.5 B	60.27 A	37.33 B	38.28 B
	***	***	***	***	***

TPC: Total phenol content, AA: Antioxidant activity, TMAC: Total monometric anthocyanin, TFC: Total flavonoid content, ***: significant at P<0.001

Total anthocyanins were calculated as cyanidin-3-glucoside and expressed in terms of milligrams of cyanidin-3-glucoside per 100 mL of fruit juice.

Ascorbic acid content in fruit juices was estimated using the volumetric method. For this purpose, juices were titrated with potassium iodate, in which starch was used as an indicator. Results were given in mg 100 mL⁻¹ [26] (Mertoğlu and Evrenosoğlu, 2019).

Microorganisms, microbial cultures and antimicrobial activities of cultivars. Five common microorganisms were used to assess the antimicrobial activity including the gram-positive *Staphylococcus aureus* (ATCC 29213), gram-negative *Escherichia coli* (ATCC 25922), *Enterococcus faecalis* (ATCC 29212) and *Candida albicans* (ATCC 14053), *Candida parapsilosis* (ATCC 22019) yeasts. The well-diffusion method was used to detect the antimicrobial activities of the juices. Equidistant holes were made in the agar using sterile cork borers (No.9, Ø 11 mm). 100 µL of blackberry juices were added to the holes using a pipettor. Dishes injected with yeasts were incubated at 25 °C for 48 h and bacterias were incubated at 37 °C for 24 h [18]. At the end of the period, inhibition zones formed on the Müller Hilton Agar were measured as mm. Anti-microbial characteristics of cultivars are expressed as ineffective (Inhibition zone < 5.5 mm), + (low effective - 5.5 mm < Inhibition zone < 10 mm), ++ (medium effective - 10.1 mm < Inhibition zone < 16 mm) and +++ (high effective - Inhibition zone > 16 mm). Developing inhibition zones were compared with those of reference antibiotics (Vancomycin, Cefepime, and Levofloxacin) and fungicide (Fluconazole).

Statistical analysis. Study was designed in accordance with the completely randomized experimental design with three replicates. The presence of statistical differences in the investigated properties of blackberry cultivars were investigated using the one-way ANOVA procedure in the Minitab-17 package program. Differences between cultivars were revealed using the Tukey-HSD multiple comparison test. Pearson correlation coefficients

were used to determine the relationships between the parameters of varieties [27].

RESULTS AND DISCUSSION

Results about the phytochemical properties of blackberry varieties are given in Table 2. All investigated characteristics were found to differ between varieties at a statistical level. In terms of total phenol content, Bursa 1 variety (1042.5 mg 100 mL⁻¹) was found to be much superior, while there was no statistical difference among other varieties. In addition, Bursa 1 has been found to be prominent in terms of antioxidant activity (63.73%) and followed by Bursa 2 (52.84%), Chester (52.5%) and Jumbo (47.57%), respectively.

Total anthocyanin and flavonoid contents of the cultivars were determined within the limits of 30.08 - 60.27 mg cyanidin-3-glucoside 100 mL⁻¹ and 29.05 - 44.13 mg CTE 100mL⁻¹, respectively. Lowest values in terms of these properties were obtained from Bursa 2 with 30.08 mg cyanidin-3-glucoside and 29.05 mg CTE 100 mL⁻¹ respectively. However, highest amount of vitamin C was also quantified from Bursa 2 (53.04 mg 100 mL⁻¹). Whereas, Chester variety stands out with its high total anthocyanin content (60.27 mg cyanidin-3-glucoside 100 mL⁻¹), similar result was obtained from Jumbo for total flavonoid content (44.13 mg CTE 100 mL⁻¹) and Bursa 1 was found to have the lowest vitamin C content (18.20 mg 100 mL⁻¹).

In a different study conducted with the same varieties, ranking of varieties in terms of anthocyanin content was found as Bursa 1 (54.82 mg cyndn-3-glucoside 100 mL⁻¹) < Bursa 2 (55.06 mg cyndn-3-glucoside 100 mL⁻¹) < Chester (80.66 mg cyndn-3-glucoside 100 mL⁻¹) < Jumbo (87.07 mg cyndn-3-glucoside 100 mL⁻¹). Furthermore, total flavonoid and phenol contents were determined in the range of 29.1 (Bursa 2) – 82.2 (Jumbo) mg CTE 100 mL⁻¹ and 2279.8 (Chester) - 2786.8 (Jumbo) mg GAE 100 g⁻¹, respectively [28]. During 2017 and 2018, another study was carried out by Balci and Keleş [29], and noted that the lowest vitamin C

TABLE 3
Anti-microbial effects of investigated blackberry cultivars

	<i>Escherichia coli</i>	<i>Enterococcus faecalis</i>	<i>Staphylococcus aureus</i>	<i>Candida albicans</i>	<i>Candida parapsilosis</i>
Bursa 1	+	++	+++	+	+
Bursa 2	++	+++	+	+	+++
Jumbo	+	+	+++	+++	+
Chester	Ineffective	+++	++	+++	Ineffective
Vancomycin	++	+++	+++	-	-
Cefepime	++	++	++	-	-
Levofloxacin	++	+++	++	-	-
Fluconazole	-	-	-	++	+

+: Low effective, ++: Moderate effective, +++: High effective

content was determined in Bursa 1 (29.34 and 11.62 mg 100 g⁻¹) and the highest in Bursa 2 varieties (59.18 and 85.13 mg 100 g⁻¹) in both years. Capacity of blackberry varieties to remove DPPH radicals were reported to vary within the limits of 74.9 - 82.07% [30] and 75.70 - 85.60% [31].

It is determined that obtained results of the present study are largely in line with the previous studies. Although variety is the most decisive factor on these features, differences in climate and soil characteristics, geographical situation of the area, type and time of harvest, storage or processing of the crops, method or periodic differences of the applied cultural processes cause significant differences on the amount of the phytochemical characteristics [26, 32, 33, 34]. Previous studies suggested that the change in ecological and growig conditions changes in phytochemical properties of varieties [35, 36, 37].

Findings about anti-microbial effects of the investigated blackberry varieties are given in Table 3. Chester variety was found to be ineffective against *Escherichia coli* bacterial strain, while the Bursa 2 variety was noted to be equivalent to the antibiotics of Vancomycin, Cefepime and Levofloxacin used as reference. On the other hand, Bursa 1 and Jumbo were found at low impact.

Enterococcus faecalis was determined as highly susceptible to Vancomycin and Levofloxacin, and moderately susceptible to Cefepime. Among the varieties, Bursa 2 and Jumbo had the same effect with Vancomycin and Levofloxacin, whereas Bursa 1 had the same effect with Cefepime and effectiveness of the Chester variety was found to be low.

Bursa 1 and Jumbo varieties were determined to be as effective as Vancomycin (high effective) against the *Staphylococcus aureus* bacterial strain while, Chester and Bursa 2 have medium and low effect, respectively.

Fluconazole fungicide which was used as reference, was found to be moderately and lowly effective against *Candida albicans* and *Candida parapsilosis*, respectively. Although Chester was

determined highly effective against *Candida albicans*, it was found ineffective to *Candida parapsilosis*. Bursa 2 and Jumbo varieties gave opposite results in terms of anti-fungal effect. Jumbo, which was found to have a high effect against *Candida albicans*, had a low effect against *Candida parapsilosis*. On the contrary, Bursa 2, was found to have a low effect against *Candida albicans*, while it was highly effective to *Candida parapsilosis*. Effectiveness of Bursa 1 variety on both yeasts was found to be low.

Salaheen et al. [10] reported that blackberry, had a restrictive effect on the development of the *Campylobacter jejuni* bacteria by lowering its virulence. In a different study, it was determined that blackberry juices prevent the development of food-borne disease factors such as *Listeria monocytogenes*, *Salmonella typhimurium* and *Escherichia coli* (O157: H7) [38]. Blackberry maintains product stabilization and improves health effects with these aspects.

Results of the correlation analysis conducted for the determination of the relations between the characteristics are given in Table 4. In line with the results, it was found that although antioxidant activity had a high level of positive relationship with total phenol (0.88***), it was negatively correlated with vitamin C (-0.51*) and total flavonoid amount (-0.58**). Phenolic compounds are synthesized in the epidermal tissues, in the phenyl propanoid pathway, and converted into each other within the plant if needed. For this reason, some phenolic compounds which are indicated to show high antioxidant activity, can be identified in some cases in a negative relation with antioxidant activity. As a matter of fact, Sariburun et al [28] reported that there was a positive correlation (0.72) between total phenol and antioxidant activity in blackberries and raspberries, although there was a negative correlation (-0.34) between total flavonoid amount and antioxidant activity. Anthocyanins are among flavonoid group compounds and the high positive correlation (0.70***) detected between these two characteristics in the study is coincide with this situation.

TABLE 4
Correlation coefficients among investigated properties

	AA	TPC	TMA	TFC	Vitamin C
TPC	0.88***				
TMA	Ns	Ns			
TFC	-0.58**	Ns	0.70***		
Vitamin C	-0.51*	-0.79	Ns	Ns	
<i>Escherichia coli</i>	Ns	Ns	-0.83***	Ns	0.40*
<i>Enterococcus faecalis</i>	0.41*	Ns	Ns	-0.56**	ns
<i>Staphylococcus aureus</i>	Ns	0.53**	0.48*	0.53**	-0.72***
<i>Candida albicans</i>	-0.52**	-0.45*	0.88***	0.90***	ns
<i>Candida parapsilosis</i>	Ns	Ns	-0.86***	-0.45*	0.52**

TPC: Total phenol content, AA: Antioxidant activity, TMA: Total monometric anthocyanin, TFC: Total flavonoid content, *, **, ***: Significant at P<0.05, 0.01 and 0.001, respectively.

Ascorbic acid, an active ingredient of Vitamin C, is one of the compounds with antioxidant property. However, it can be detected in negative relation with antioxidant activity especially in berry fruits that are rich in terms of phenolic compounds [39]. This is thought to be due to the fact that phenolic compounds contribute mainly to antioxidant activity and the amount of ascorbic acid is much lower than these compounds. In addition, total phenol content, which has a positive relation with antioxidant activity, was determined in a negative relationship with vitamin C (-0.79). Obtained results were found to be compatible with previous studies carried out with berries [39, 40].

Relations of phytochemical properties with anti-microbial properties were varied. Vitamin C was found to have a restrictive effect on the development of *Escherichia coli* (0.40*) and *Candida parapsilosis* (0.52**). All of the phenolic compounds had inhibitory effect on the development of *Staphylococcus aureus* and correlation coefficient of inhibition zone with total phenol, anthocyanin and flavonoid properties were found as 0.53**, 0.48*, 0.53**, respectively. Increase of total anthocyanin (0.88***) and phenol (0.90***) properties also had a restrictive effect on the development of *Candida albicans*. Preventive effect on many different microorganisms were reported in different researches carried out with blackberry.

CONCLUSIONS

In line with obtained results, Bursa 1 cultivar stands out in terms of total phenol content (1042.5 mg 100 mL⁻¹) and antioxidant activity (%63.73), while Bursa 2 was found to be superior in terms of vitamin C amount with 53.04 mg 100 mL⁻¹. Similar findings were obtained from Chester (60.27 mg cyndn-3-glucoside 100 mL⁻¹) and Jumbo (44.13 mg Catechin 100 mL⁻¹) for monometric anthocyanin content and total flavonoid content, respectively.

Due to the disease agents' resistance to active ingredient and the negative effects of synthetic drugs, community individuals and scientists have

turned their faces to natural resources. In this study, Bursa 2 was determined to be more effective than the active ingredients used as a reference against to *Escherichia coli*, *Enterococcus faecalis* and *Candida parapsilosis*. Same effects were seen in Jumbo to *Candida albicans* and *Staphylococcus aureus*; in Chester to *Enterococcus faecalis* and *Candida albicans* and in Bursa 1 to *Staphylococcus aureus*.

It can be said that it will be beneficial to periodically consume fresh or processed blackberry products in terms of adequate, balanced and healthy nutrition thanks to their rich phytochemical content and strong anti-microbial properties.

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