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Phytochemical Investigation, Antioxidant, and Enzyme Inhibitory Activities of Blackberry (*Rubus fruticosus*) Fruits

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Abstract

This scientific paper explores the phytochemical composition of blackberry (*Rubus fruticosus* L.) fruit and investigates its antioxidant and enzyme inhibitory activities. Blackberry is known for its rich nutritional profile and potential health benefits, making it a subject of interest in the field of functional foods and natural medicine. The study aims to provide valuable insights into the bioactive compounds present in blackberry fruit, their antioxidant properties, and their potential as enzyme inhibitors. According to the results, it was determined that the fruit extracts had strong antioxidant and moderate enzyme inhibition activity. HPLC analysis showed that the fruit extract generally contained *o*-coumaric acid (7.74-30.87), procatechin (16.44 and 36.91 in BE and BA, respectively), ellagic acid (0.51-3.03) and quercetin (0.22-8.50) as major components.

Key Words: Blackberry, phytochemicals, antioxidant activity, enzyme inhibition, functional foods, HPLC

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1. Introduction

Antioxidants are compounds that help neutralize harmful molecules called free radicals. Free radicals are produced naturally in the body as part of normal metabolic processes, but they can also be generated in higher amounts due to factors like stress, pollution, unhealthy diet, and certain medical conditions (Lobo et al., 2010). Antioxidants help protect cells and tissues from oxidative damage, thereby reducing the risk of complications associated with diabetes. For example, antioxidants such as vitamin C, vitamin E, and beta-carotene can help preserve eye health and reduce the risk of diabetic retinopathy(Dal & Sigrist, 2016). The relationship between antioxidants and Alzheimer's disease revolves around oxidative stress, inflammation, and the damage caused by reactive oxygen species (ROS) in the brain (Manoharan et al., 2016).

Blackberry (*Rubus fruticosus* L.), a member of the Rosaceae family, is a widely consumed fruit known for its distinctive flavor and dark color. Previous research has highlighted its nutritional content, including vitamins, minerals, and dietary fibers. Additionally, blackberries are reported to contain various phytochemicals with potential health-promoting effects (Martins et al., 2023).

Inhibition of enzymes plays an important role in the treatment of diseases such as Alzheimer's disease, diabetes and Parkinson's(Arcone et al., 2023). Some of these diseases are caused by the irregular functioning of certain enzymes in the body. For this reason, inhibition of enzymes is very important in the treatment of these diseases. Alzheimer's disease is associated with the buildup of certain proteins in the brain. A buildup of a protein called beta-amyloid can lead to the death of nerve cells and ultimately cognitive impairment. At this point, enzyme inhibition can be used to prevent beta-amyloid production or accumulation (Ibach & Haen, 2004).

Diabetes occurs when the pancreas does not produce enough insulin or the body cannot use insulin effectively. Insulin is an enzyme that helps glucose enter cells. Inhibiting the enzymes α-glucosidase and α-amylase, which are responsible for breaking down carbohydrates during digestion, can effectively lower the rise in blood glucose after meals. This makes it a crucial approach for managing blood glucose levels in individuals with type-2 diabetes and those at risk. Recently, there has been a resurgence of interest in plant-based medicines and functional foods that can modify physiological responses, aiming to prevent and treat diabetes and obesity (Tundis et al., 2010).

Parkinson's disease is characterized by damage or loss of dopamine-producing cells in the brain. Dopamine is a neurotransmitter that plays an important role in movement control and emotional reactions. In the treatment of Parkinson's disease, studies are being conducted on the inhibition of enzymes to increase dopamine production or reduce dopamine destruction(Nagatsu et al., 2022). There are studies in the literature on the analysis of the antioxidant and phytochemical content of blackberry fruit. However, previous studies were carried out on a single type of extract, and no additional studies on enzyme inhibition activity were found. Therefore, this study aimed to investigate the phytochemical content, antioxidant and enzyme inhibition of blackberry fruit, which is frequently consumed in our daily lives. In this way, the beneficial effects of blackberry fruit on health will be evaluated and the basis will be formed through scientific study for the development of nutritional supplements derived from blackberry.

2. Material and Methods

2.1. Sample Collection and Preparation

Fresh blackberry fruits were harvested from a local orchard (From Konya province, Turkiye) and immediately transported to the laboratory. The fruits were cleaned, and freeze-dried to preserve their phytochemical composition.

2.2. Preparation of extracts

The freeze dried samples were cut into small pieces and soaked seperately in 50% acetone, 80% ethanol and 80% Methanol containing 0.01% HCl at ambient tempereture for 24h. The extract were filtered and concentrated by a rotary evaporator to obtaine dry acetone(BA), ethanol(BE) and methanol (BM) extracts, respectively.

2.3. Phytochemical Analysis

The phytochemical profile of blackberry was determined using various extraction techniques, including solvent extraction and chromatographic methods. Common phytochemicals such as flavonoids (Quettier-Deleu et al., 2000), phenolic acids (Clarke et al., 2013), and anthocyanins (Fredes González et al., 2014) were quantified by septrophotometric method. The phenolic profiling was also determined with HPLC (Fig. 1).

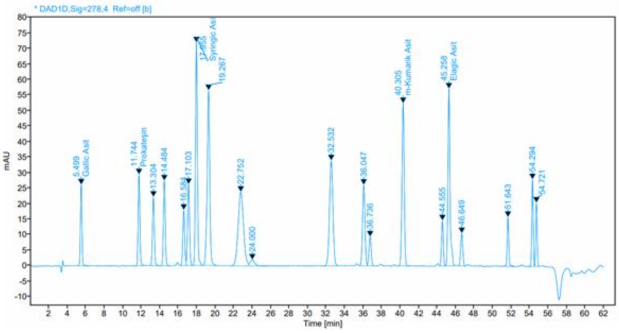

Figure 1. HPLC chromatogram of standard phenolic compounds

2.4. Antioxidant Activity Assays

The antioxidant potential of blackberry extracts was assessed using standard assays, including the 2,2-diphenyl-1-picrylhydrazyl (DPPH)(Wang et al., 2008) and ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (Chun et al., 2005) radical scavenging assay.

2.5. Enzyme Inhibitory Assays

Enzyme inhibitory activities, against key enzymes involved in various metabolic processes, such as α-amylase (Yang & Kong, 2016) and α-glucosidase (Bhatia et al., 2019) for carbohydrate metabolism, tyrosinase for skin whitening effect, and acetyl-, butyrylcholinesterase for neurological health (Šinko et al., 2007) were performed according to previously reported experimental methods.

The cholinesterase inhibitory activity (AChE and BChE) of the extracts was conducted using Elmann's method. The α-amylase activity was determined using starch as a substrate in a colorimetric reaction using 3,5-dinitrosalicylic acid. The α- glucosidase activity was determined using para-nitrophenyl-α- d-glucopyranoside as a substrate. Tyrosinase inhibitory was performed using L-DOPA as the substrate (Jeong et al., 2009). The tests were conducted via distribution of 140 μL of a solution of mushroom tyrosinase (250 U/mL in phosphate buffer) in each well, together with 25 μL of the extracts evaluated. Kojic acid was used as a positive control and DMSO alone was used as the negative control.

3. Results and Discussion

The present study was conducted to determine total phenol, flavonoid, anthocyanin content as well as the in vitro antioxidant and enzyme inhibitory activity of blackberry fruits.

3.1. Phytochemical Composition

Table 1 presents the findings on the total phenolic and flavonoid contents, as well as total anthocyanin content of various extracts of blackberry fruits.

Table 1. Total anthocyanin, flavonoid and phenolic content of blackberry acetone, ethanol and methanol extracts

Specimens	Extract yield (%, g/g)	Total anthocyanins (mg of Cyanidin-3-Glucoside E/g)	Total flavonoids (mg of QE/g)	Total phenolics (mg of GAE/g)
BM	11.05	4.828 ± 0.001	0.03± 0.008	25.37±1.13
BA	13.85	3.374 ± 0.081	1.95±1.08	36.62±0.59
BE	10.44	5.997 ± 0.003	2.18 ± 0.07	23.76±0.85

The total anthocyanin content of methanol, acetone and ethanol extract were 4.828 ± 0.0001, 3.374 ± 0.081 and 5.997 ± 0.003 mg/g cyanidin 3-glucoside equivalent, respectively. The acetone extract had the highest TPC (36.62±0.59 mg/g GAE), followed by BM extract (25.37±1.13 mg/g GAE) and BE extract (23.76±0.85 mg/g GAE). The total flavonoid content of methanol, acetone and ethanol extract were

0.03 ± 0.008, 1.95±1.08 and 2.18 ± 0.07 mg/g quercetin equivalent, respectively. In a previous study, TPC, TFC and TAC of blackberry were found as 5.58 mg GAE/g, 11.83 mg RE/g and 3.99 mg catechin/g dry weight, respectively (Huang et al., 2012). In another study, TPC and TFC were found to be 87.25 mg GAE/g and 48.97 mg CE/g, respectively (Tan & Chang, 2017). HPLC analysis of phenolic compounds was performed by determining As can be seen from the HPLC result (Fig. 2-4), there is a difference in the profile of phenolic compounds found in the extracts.

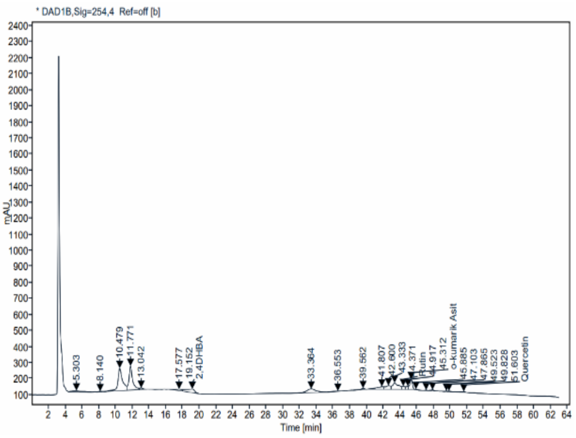

Figure 2. HPLC chromatogram of blackberry acetone extract

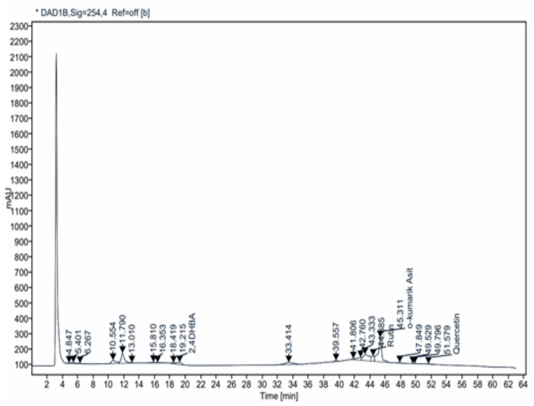

Figure 3. HPLC chromatogram of blackberry ethanol extract

While o-coumaric acid was found in all extracts (Table 2), the highest level was detected in methanol and ethanol extracts. Procatechin was found to be highest in acetone extract.

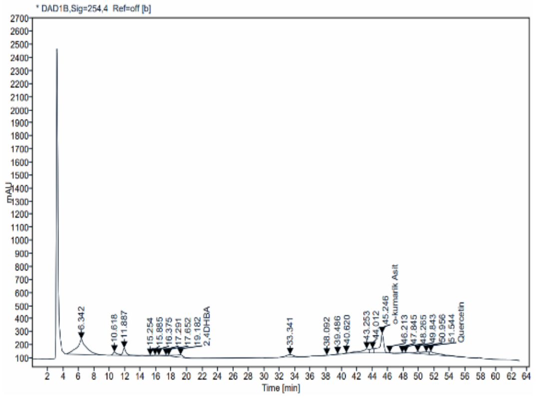

Figure 4. HPLC chromatogram of blackberry methanol extract

Table 2. Phenolic content of blackberry fruit extracts by HPLC j (µg/mg)

Name	RT (min)	BA	BE	BM
Gallic acid	5.499	-	-	-
Procatechin	11.744	36.909	16.443	-
Chlorogenic acid	13.304	-	-	-
4-hydroxy benzoic	14.484	3.039	-	-
2,5- dihydroxy	15.914	-	-	-
Vanilic acid	16.584	-	-	-
Cafeic acid	17.103	-	-	-
Syringic acid	17.955	-	-	-
2,4- dihydroxy	19.088	-	1.217	2.562
Vanilin	19.270	0.768	0.435	0.711
4H1,3 - benzoic acid	22.752	-	-	-
2,3- dihydroxy	24.011	-	-	-
p-Coumaric acid	32.532	-	-	-
Ferulic acid	36.047	-	-	-
Sinapic acid	36.736	-	-	
m-coumaric acid	40.305	-	-	0.200
Rutin	44.555	3.995	7.077	-
Elagic acid	45.258	0.508	1.571	3.029
o-coumaric acid	45.557	7.744	27.990	30.873
Salisilic acid	46.649	--	-	-
Quercetin	51.643	0.375	0.223	8.504
Caempherol	54.294	-	-	-
Apigenin	54.721	-	-	-

3.2. Antioxidant Activity

According to the antioxidant activity results of blackberry fruit extracts, a proportional increase in radical scavenging activity was observed depending on the increase in extract concentration. When compared at the low concentration (75 µg/mL), acetone extract showed higher activity in terms of both DPPH (55.45%) and ABTS (72.45%) radical scavenging activity (Fig. 2, 3).

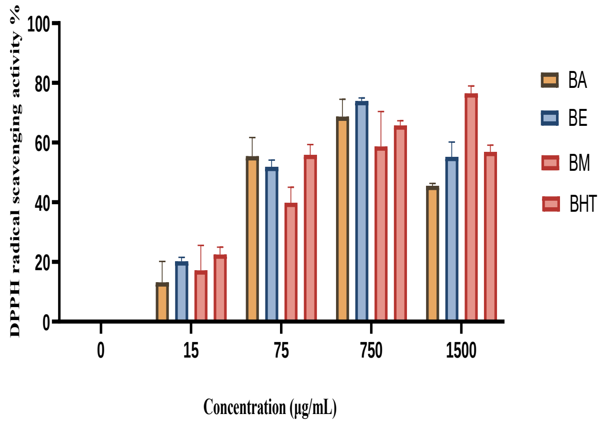

Figure 5. DPPH radical scavengign activity of blackberry acetone, ethanol and methanol extracts

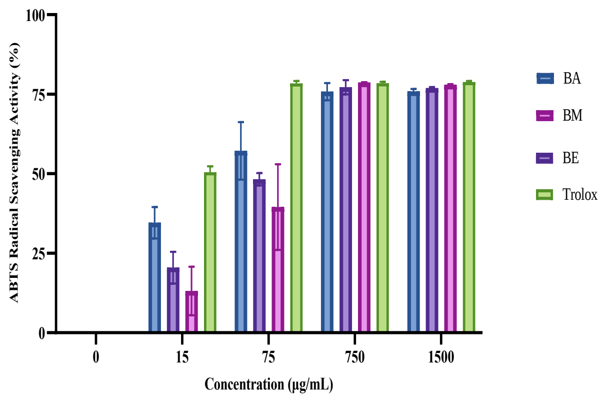

Figure 6. ABTS radical scavengign activity of blackberry acetone, ethanol and methanol extracts

The DPPH radical scavenging activity of BA, BE and BM extracts shown to be IC_{50} value of 319.6± 0.34, 179.9±0.40 and 235.1±0.52 µg/mL, respectively. The ABTS radical scavenging activity of BA, BE and BM extracts shown to be IC_{50} value of 69.01± 0.50, 115.3±0.60 and 157.0±0.71 µg/mL, respectively. Blackberry extracts demonstrated significant antioxidant activities in all tested assays, suggesting the potential for free radical scavenging and reducing oxidative stress. The DPPH and ABTS radical scavenging potential of different blackberry cultivars methanol extract were showed 108.43-146.89 mg TE/g and 126.00-177.11 mg TE/g, respectively (Sariburun et al., 2010).

3.3. Enzyme Inhibitory Activities

The inhibitory effects of blackberry extracts on α-amylase, α-glucosidase, and acetylcholinesterase activities will be discussed, highlighting their potential role in managing diabetes and supporting cognitive function. In general, acetone extract showed higher AChE (10.11%), BChE (15.58%), α-amylase (25.84%) inhibitory activity while methanol extract demonstrated greater α-glucosidase (588.46) and tyrosinase (58.62%) inhibitory activity followed by ethanol extract (Table 3). In a study, blackberry crude extract showed inhibitory activity against α-amylase and α-glucosidase with the IC_{50} value of 1.56 and 57.03 mg/ml, respectively (Tan & Chang, 2017). Polyphenols from berries have the potential to modulate starch digestion and berry components can be substitute for acarbose or used as pharmaceutical enzyme inhibitor (Boath et al., 2012).

Table 3. Enzyme inhibitory activity of blackberry acetone, ethanol and methanol extracts (%inhibition at 1 mg/ml concentration)

Specimens	AChE	BChE	α-amylase	α-glucosidase	Tyrosinase
BM	7.19±3.26	7.19±3.26	13.29±4.43	88.46±0.18	58.62±3.00
BA	10.11±3.33	15.58±1.16	25.84±1.55	70.52±0.57	54.14±1.70
BE	4.01±1.65	10.73±2.15	12.85±5.03	83.95±2.05	56.71±5.62
Galanthamine	33.09±2.78	20.20±3.37	-	-	-
Acarbose	-	-	39.72±4.44	70.45±4.51	-
Kojic acid	-	-	-	-	61.96±1.45

The results of this analysis indicated that it is important to point out that blackberry fruit is an excellent raw material for obtaining biologically valuable products. As far as we are concerned, this is the first study to investigate phytochemical profiling and enzyme inhibition activity of black berry fruits with different extracts.

4. Conclusion

This study provides a comprehensive analysis of the phytochemical composition of blackberry fruit and its antioxidant and enzyme inhibitory activities. The findings suggest that blackberry may serve as a valuable source of bioactive compounds with potential health benefits. Further research is warranted to explore the therapeutic applications of blackberry in preventing or managing various health conditions.

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Author Contribution

Conceptualization, A.S. and N.E.; methodology, software, validation, and formal analysis, N.E.; data curation, A.S.; writing—original draft preparation, A.S.; writing-review and editing, visualization, and supervision, N.E.; funding acquisition, N.E. All authors have read and agreed to the published version of the manuscript..

Conflicts of Interest

The authors declare no conflicts of interest.

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