Turk J Pharm Sci 2019;16(2):175-183
DOI: 10.4274/tjps.galenos.2018.28247

ORIGINAL ARTICLE

Investigation of the Antioxidant, α-Glucosidase Inhibitory, Anti-inflammatory, and DNA Protective Properties of *Vaccinium arctostaphylos* L.

Vaccinium arctostaphylos L.'nin Antioksidan, α-Glukozidazı İnhibe Edici, Anti-inflamatuvar ve DNA Koruyucu Özelliklerinin İncelenmesi

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ABSTRACT

Objectives: The scope of this study was to investigate the total phenolic, anthocyanin, and flavonoid contents and the biological properties of ethanol extract (EE), methanol extract (ME), and aqueous extract (AE) from *Vaccinium arctostaphylos* L.

Materials and Methods: EE, ME, and AE of *V. arctostaphylos* were prepared. Various biological activities such as total phenolic, anthocyanin, and flavonoid contents, and antioxidant (2,2'-diphenyl-1-picrylhydrazyl ferrous ion-chelating, and ferric reducing antioxidant power assays), α-glucosidase inhibitory, anti-inflammatory, and DNA protective properties of these extracts were studied.

Results: EE exhibited the highest total phenolic, anthocyanin, and flavonoid contents with 44.42±1.22 mg gallic acid equivalents/g dry weight, 8.46±0.49 mg/Cyaniding-3-glucoside equivalents/g dry weight, and 9.22±0.92 mg quercetin equivalents/g dry weight, respectively. The antioxidant activities of the extracts followed the order: EE>ME>AE. EE and ME inhibited α-glucosidase enzyme and their IC_{50} values were 0.301±0.002 mg/mL and 0.477±0.003 mg/mL, respectively. In addition, EE and ME were determined as noncompetitive inhibitors with inhibitory constant (K_i) values of 0.48±0.02 mg/mL and 0.46±0.01 mg/mL, respectively. EE in 100 and 300 mg/kg doses caused a significant reduction in formalin-induced edema in mice, demonstrating the anti-inflammatory effect of EE. In DNA protective studies, all of the extracts protected supercoiled plasmid pBR322 DNA against damage caused by Fenton's reagents due to their radical scavenging activities.

Conclusion: Our results demonstrated that EE of *V. arctostaphylos L.* had strong antioxidant, anti-inflammatory, α-glucosidase inhibitory, and DNA protective effects, suggesting that it might be an effective medical plant to prevent or treat diseases associated with oxidative damage and inflammation.

Key words: Antioxidant, anti-inflammatory, DNA, α-glucosidase, *Vaccinium arctostaphylos*

ÖZ

Amaç: Bu çalışmanın amacı *Vaccinium arctostaphylos* L.'den hazırlanan etanol (EE), metanol (ME) ve su (AE) ekstraktlarının toplam fenolik, antosiyanin, flavonoit içerikleri ve biyolojik özelliklerinin incelenmesidir.

Gereç ve Yöntemler: *V. arctostaphylos*'un EE, ME ve AE ekstraktları hazırlanmıştır. Bu ekstraktların total fenolik, antosiyanin ve flavonoid içerikleri, antioksidan (2,2'-difenil-1-pikrilhidrazil, metal iyon şelatlama ve ferrik indirgeyici antioksidan gücü metotları), α-glukozidaz, anti-inflamatuvar ve DNA koruma özellikleri araştırılmıştır.

Bulgular: EE, 44.42±1.22 mg galik asit eşdeğeri/g kuru ağırlık, 8.46±0.49 mg/siyanidin-3-glukozid eş değerleri/g kuru ağırlık ve 9.22±0.92 mg quercetin eş değerleri/g kuru ağırlık değerleriyle en yüksek toplam fenolik, antosiyanin ve flavonoid içeriğine sahip olduğu görülmüştür. Bununla birlikte ekstraktların antioksidan aktiviteleri sırasıyla EE>ME>AE olduğu belirlendi. EE ve ME α-glukozidaz enzimini sırasıyla 0.301±0.002 mg/mL ve 0.477±0.003 mg/mL IC_{50} değerleriyle inhibe etmiştir. Ayrıca, EE ve ME'nin inhibisyon sabiti (K_i) değerleri 0.48±0.02 mg/mL ve 0.46±0.01 mg/mL bulunarak, yarışmasız inhibisyon gerçekleştirdikleri belirlenmiştir. EE'nin 100 ve 300 mg/kg dozları farelerde formalin ile indüklenen ödemi önemli derecede azalttığı belirlenmiştir. DNA koruma çalışmalarında, ekstraktlar radikal süpürme aktivitesinden dolayı Fenton reaktifiyle oluşturulan hasara karşı süpersarmal plasmid pBR322 DNA'yı korumuştur.

Sonuç: Sonuçlarımız, *V. arctostaphylos* L.'nin EE'sinin güçlü antioksidan, anti-inflamatuvar, α-glukozidaz inhibisyon ve DNA koruyucu etkilere sahip olduğunu göstermiştir; bu, oksidatif hasar ve iltihaplanma ile ilişkili hastalıkları önlemek veya tedavi etmek için etkili bir tıbbi bitki olabileceğini düşündürmektedir.

Anahtar kelimeler: Antioksidan, anti-inflamatuvar, DNA, α-glukozidaz, *Vaccinium arctostaphylos*

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Received: 14.02.2018, **Accepted:** 15.03.2018

INTRODUCTION

Medicinal plants containing secondary metabolites such as phenolic, anthocyanin, and flavonoid compounds have been used as alternative therapeutic tools to treat many diseases throughout medical history.[1] Many plants are considered able to scavenge and hinder free radicals, including reactive oxygen species (ROS) such as hydroxyl radical ($OH^{\cdot}$), hydrogen peroxide (H_2O_2), and superoxide anion radical ($O_2^{\cdot-}$), which induce oxidative damage in biomolecules due to these secondary metabolites possessing antioxidant activity.[2] In addition, plant-based natural antioxidants are preferred to synthetic ones due to their good safety profiles.[3] Therefore, there is growing interest in finding natural compounds that could prevent oxidative damage underlying the pathogenesis of many diseases.

The genus *Vaccinium* belongs to the family *Ericaceae*; it includes approximately 450 species distributed in the Northern Hemisphere and tropical mountains of America and Asia.[4,5] Numerous studies have reported that *Vaccinium* possesses several biological and pharmacological activities, making it an attractive medical plant.[6] Previous studies reported that *Vaccinium* species have been used for memory improvement, eyesight protection, cardiovascular protection, and for their antioxidant, antidiabetic, and anticancer activities.[7-10]

Vaccinium arctostaphylos L., commonly named the Caucasian whortleberry, is the only member of the genus *Vaccinium* and is widely used as an antidiabetic and antihypertensive agent.[11,12] To date, this plant has been reported to contain phenolic compounds such as anthocyanin, flavanol, and procyanidins that are responsible for numerous biological activities such as reducing serum glucose concentration and improving lipid profile, antioxidant and urinary antiseptic activities, etc.[12,13] Ayaz reported that delphinidin, petunidin, and malvidin were the most predominant anthocyanins of *V. arctostaphylos* L. fruits, while caffeic acid and *p*-coumaric acid were the major phenolic compounds.[14,15]

Diabetes mellitus (DM) is one of the most prevalent metabolic disorders, characterized by hyperglycemia triggered by inherited and acquired formation of insulin or by insulin resistance.[16,17] According to the International Diabetes Federation, 425 million people are living with DM; this number is expected to increase to 629 million by 2045 approximately. In addition, 352 million adults are at risk of developing DM.[18]

α-Glucosidase (EC 3.2.1.20) catalyzes the break of the glycosidic bond in oligosaccharides into α-glucose, resulting in postprandial hyperglycemia.[19] Thus, an α-glucosidase inhibitor could be useful to treat obesity and DM. Commercial α-glucosidase inhibitors such as acarbose, voglibose, and miglitol are currently used against DM, but many adverse effects have been observed such as abdominal pain, renal tumors, hepatic injury, diarrhea, and flatulence.[20] Therefore, scientists seek novel natural α-glucosidase inhibitors against DM.

To the best of our knowledge, there is no report on kinetic studies of the α-glucosidase inhibition, anti-inflammatory, and DNA protective properties of *V. arctostaphylos*. The goal of the present study was to evaluate the antioxidant, anti-inflammatory, α-glucosidase inhibitory, and DNA protective properties of ethanol extract (EE), methanol extract (ME), and aqueous extract (AE) of *V. arctostaphylos* L. from Turkey.

EXPERIMENTAL

Plant material and sample preparation

V. arctostaphylos fruits were collected from Uzungöl, Trabzon, Turkey, in August 2013 and identified by Prof. Kamil Coşkunçelebi. The fruits were dried at room temperature for 2 weeks and the dried samples were pulverized using an automatic herbal grinder. Then the pulverized fruits were extracted with solvent (ethanol, methanol, and water) in a shaker for 6 h×3. After shaking, the mixtures were filtered with Whatman filter paper No: 1. The solvent was evaporated under reduced pressure by a Heidolph Hei-VAP rotary evaporator. The extracts were kept +4°C until further use.[21]

Total phenolic content

The total phenolic content of extracts was evaluated using the Folin–Ciocalteu reagent method described by Keser. The calibration curve was obtained with gallic acid (GA) and the results expressed as mg gallic acid equivalents (GAE) per g dry weight of the sample.[22]

Total anthocyanin content

The total anthocyanin content of extracts was determined with the pH differential absorbance method, as described by Cheng and Breen, and expressed as μg cyaniding-3-glucoside equivalents (CGE) per g dry weight of the fruit.[23]

Total flavonoid content

The total flavonoid content of extracts was investigated using an $Al(NO_3)_3$ assay and expressed as mg quercetin equivalents (QEE) per g dry weight of the sample.[24]

Antioxidant activities

2,2-diphenyl-1-picrylhydrazyl radical scavenging assay

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activities of extracts were investigated using the method described by Blois and the inhibition percentage was calculated using Formula 1.[25]

$A_{control}$ is the antioxidant activity without extracts and $A_{extract}$ is the antioxidant activity with extracts at various concentrations. SC_{50} values represented the concentration of the extracts that caused 50% inhibition of radical formation. GA was used as a positive control.

Ferrous ion-chelating assay

The ferrous ion-chelating activity of the extract was investigated using Chua et al.'s[26] method and the ferrous ion chelating capacities were calculated using Formula 1.

Ferric reducing antioxidant power assay

The ferric reducing antioxidant power (FRAP) effects of extracts were evaluated using the method described by Oyaizu and expressed as butylated hydroxyanisole equivalents (BHAE) per g dry weight of the sample.[27]

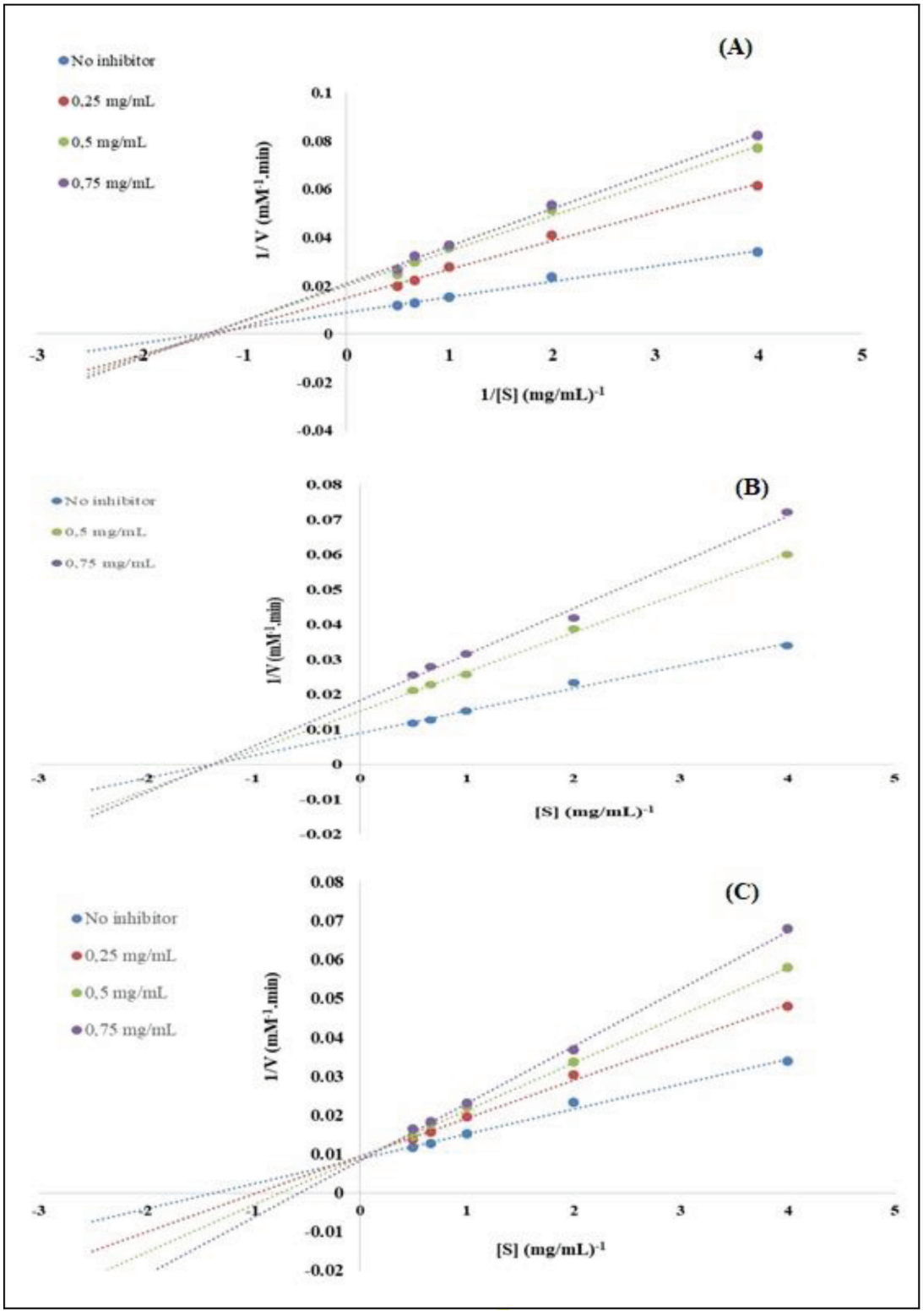

Figure 1. Lineweaver–Burk plots for kinetic analysis of α-glucosidase inhibition by a) EE, b) ME, and c) AE

EE: Ethanol extract, ME: Methanol extract, AE: Aqueous extract

Enzyme inhibition

α-Glucosidase inhibition assay

The α-glucosidase inhibitory properties were examined according to a previous study with a slight modification.[28] In the present study, the extracts and 0.5 U/mL α-glucosidase enzyme were mixed in a 96-well microplate and left to react for 10 min. After that, 5 mM 4-pNPG was added and the reaction mixture was incubated for 10 min. The absorbance was measured at

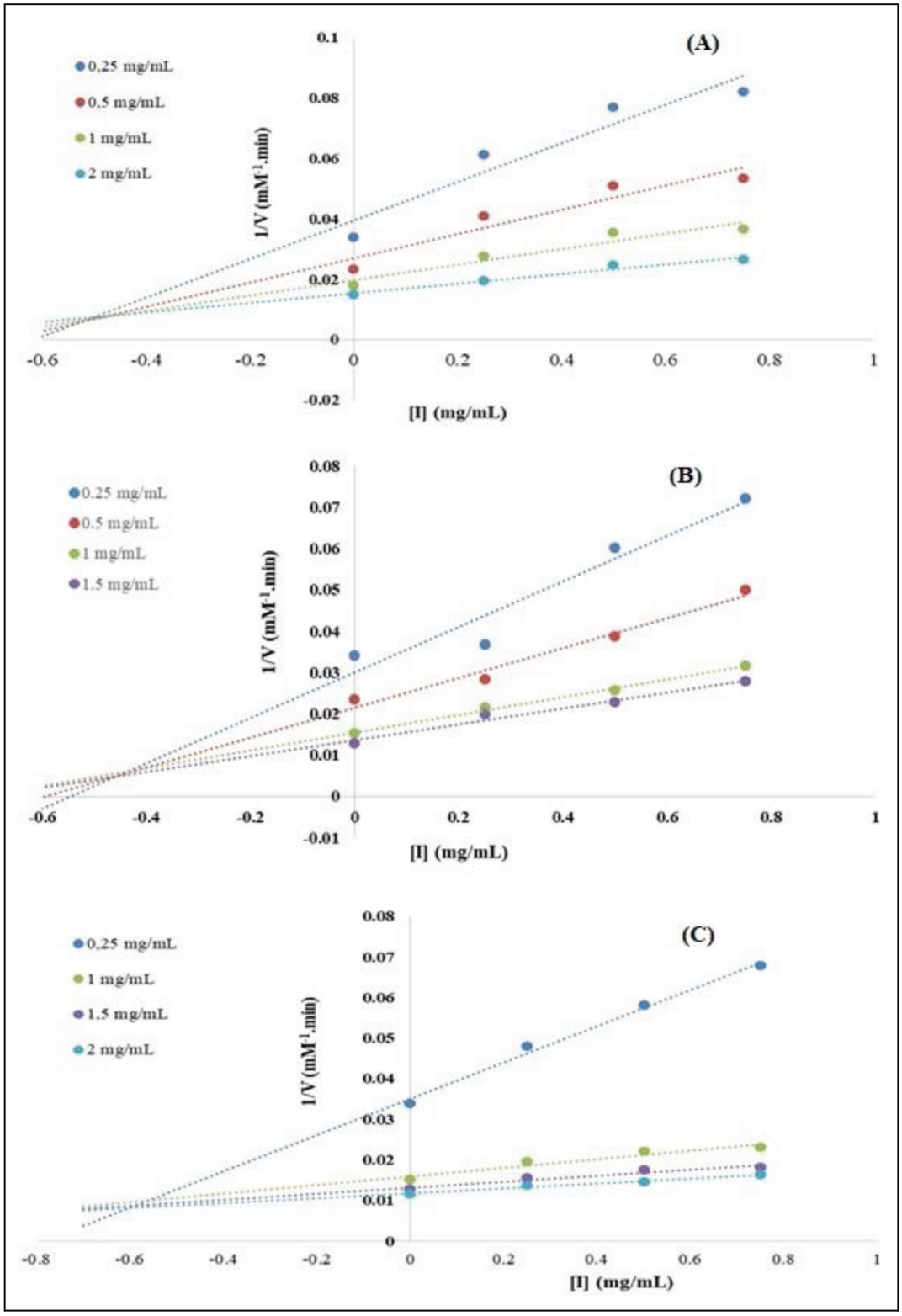

Figure 2. Dixon plot kinetic analysis of α-glucosidase inhibition by a) EE, b) ME and c) AE

EE: Ethanol extract, ME: Methanol extract, AE: Aqueous extract

405 nm using a 96-well microplate reader. Acarbose was used as a standard reference. The percentage of α-glucosidase inhibition was calculated as follows:

$$\alpha\text{– glucosidase inhibition (\%)} = \left[\frac{(A_{control} - A_{extract})}{A_{control}}\right] \times 100$$

Here $A_{control}$ is the activity of enzyme without extract and $A_{extract}$ is the activity of enzyme with extract at various concentrations.

Kinetic analysis of α-glucosidase inhibition

In order to investigate the inhibition type and inhibition constant (K_i) values of extracts, Lineweaver–Burk and Dixon plots were used against α-glucosidase enzyme.[29] The kinetic analysis was conducted with various 4-pNPG concentrations in the absence and presence of extracts.[30]

DNA protective properties

The DNA protective properties of extracts of *V. arctostaphylos* fruits against oxidative damage caused by $OH^{\cdot}$ were monitored by conversion of supercoiled plasmid pBR322 DNA to open circular form as described by Yeung et al.[31] In the present study, the total volume of the mixture was 10 µL, containing Tris-HCl buffer (pH 7.0), supercoiled plasmid pBR322 DNA, 1 mM $FeSO_4$, 2% H_2O_2, and various concentration of extracts (0.125, 0.25, and 0.5 mg/mL). The mixtures were incubated at 37°C for 1 h. After incubation, loading buffer (bromophenol, glycerol, SDS, and xylene cyanol) was added to the mixture. The mixtures were loaded on agarose gel and electrophoresis was performed at 100 V for 90 min using the wide Mini-Sub cell GT system from Bio-Rad. The results were visualized with the Bio-Rad Gel Doc XR system.[32]

In vivo anti-inflammatory activity

Animals

The male Balb/c mice (25-35 g; n=24) used in this study were kept in temperature controlled (24±1°C) rooms with food and water given *ad libitum*. They were allowed to acclimatize to the laboratory conditions for 1 week. The experiments were carried out between 9 am and 4 pm. The experimental protocol was approved by the Institutional Animal Ethical Committee of Karadeniz Technical University (2017/45).

Formalin-induced hind paw edema

The anti-inflammatory activity of EE was evaluated by formalin-induced edema. The mice were divided into the following 4 groups with 6 mice in each group: 1) control (saline, 10 mL/kg p.o.), 2) diclofenac (10 mg/kg, i.p.), 3) EE 100 mg/kg p.o., 4) EE 300 mg/kg p.o. Extract was administered orally to the mice for three consecutive days. Then 60 min after the last dose of extracts and 30 min after administration of diclofenac and saline, 20 µL of 1% formalin (in 0.9% saline) solution was injected into the dorsal surface of the right hind paws of the animals to form edema. Edema was expressed as the increment in paw thickness and was measured 30 min before and 30, 60, and 120 min after the formalin injection by micrometer caliper.[33]

Statistical analysis

The data were analyzed using GraphPad Prism 5.0 and Microsoft Excel Windows 10. *In vitro* tests were performed in triplicate and the data were expressed as the mean ± standard deviation. Statistical analysis was performed with two-way analysis of variance followed by Bonferroni tests. $P<0.05$ was considered statistically significant.[34]

RESULTS

Determination of total phenolic, anthocyanin, and flavonoid contents

The total phenolic, total anthocyanin, and total flavonoid contents of extracts are shown in Table 1. EE had the highest total phenolic, anthocyanin, and flavonoid contents, with 44.42±1.22 mg GAE/g dry weight, 8.46±0.49 mg CGE/g dry weight, and 9.22±0.92 mg QEE/g dry weight, respectively. In addition, ME had higher total phenolic, anthocyanin, and flavonoid contents than AE, about 1.63-, 1.40-, and 5.57-fold, respectively.

Evaluation of antioxidant activity

The SC_{50} values of DPPH and metal chelating radical scavenging activities of extracts are presented in Table 2. All extracts demonstrated scavenging activities against DPPH radical in a concentration-dependent manner. The DPPH radical scavenging assay showed that EE had significant antioxidant activities, with an SC_{50} value of 0.141±0.009 mg/mL. The extracts

Table 1. Total phenolic, anthocyanin, and flavonoid contents of *Vaccinium arctostaphylos* L. fruit extracts

Extracts	Total phenolic content (mg GAE/g dry weight)	Total anthocyanin content (mg CGE/g dry weight)	Total flavonoid content (mg QEE/g dry weight)
EE	44.42±1.22	8.46±0.49	9.22±0.92
ME	26.78±0.67	6.02±1.20	7.80±0.44
AE	16.42±0.15	4.29±0.33	1.40±0.02

EE: Ethanol extract, ME: Methanol extract, AE: Aqueous extract, GAE: Gallic acid equivalents, CGE: Cyaniding-3-glucoside equivalents, QEE: Quercetin equivalents

Table 2. DPPH radical scavenging, metal chelating, and FRAP activities of *Vaccinium arctostaphylos* L. fruit extracts

Extracts	DPPH (IC_{50} values mg/mL)	Metal chelating effect (IC_{50} values mg/mL)	FRAP (mg BHAE/g dry weight)
EE	0.141±0.009	0.453±0.007	62.06±2.13
ME	0.211±0.011	0.757±0.004	47.70±2.77
AE	0.263±0.003	0.909±0.006	15.39±0.98
GA	0.068±0.001	1.243±0.010	-
EDTA	-	0.020±0.001	-

EE: Ethanol extract, ME: Methanol extract, AE: Aqueous extract, GA: Gallic acid, EDTA: Ethylenediaminetetraacetic acid, FRAP: Ferric reducing antioxidant power, DPPH: 2,2-diphenyl-1-picrylhydrazyl, BHAE: Butylated hydroxyanisole equivalents

demonstrated moderate metal chelating activities compared to ethylenediaminetetraacetic acid. EE had the highest chelating activities, with an SC_{50} value of 0.453±0.007 mg/mL, whereas AE had the lowest activities, with an SC_{50} value of 0.909±0.006 mg/mL.

The FRAP activities of the extracts are presented in Table 2 and expressed as mg BHAE/g dry weight. EE had the highest reducing activities, with 62.06±2.13 mg BHAE/g dry weight, while ME and AE were 47.70±2.77 and 15.39±0.98 mg BHAE/g dry weight, respectively.

Enzyme inhibition and kinetic analysis of α-glucosidase inhibition

The α-glucosidase inhibitory effects of extracts were evaluated using the da Silva Pinto method when compared to acarbose as a standard reference. The results obtained in the present study were expressed as IC_{50} values and are presented in Table 3. The extracts demonstrated an inhibitory effect against α-glucosidase ranging from 0.301±0.003 mg/mL to 0.591±0.007 mg/mL as IC_{50} values. EE exhibited the most potent inhibitory activity against α-glucosidase, with an IC_{50} value of 0.301±0.003 mg/mL.

The kinetic analysis of extracts was carried out using Lineweaver–Burk and Dixon plots and is presented in Table 3 and Figures 1 and 2. These data obtained were plotted as 1/activity (1/V) against 1/substrate concentration (1/[S]) for Lineweaver–Burk plots. These results revealed that the inhibition type EE and ME were noncompetitive, while AE was competitive. K_i values using Dixon plots were plotted as 1/enzyme velocity versus inhibitor concentration with varying concentrations of the substrate. The K_i values of EE, ME, and AE were 0.48±0.02 mg/mL, 0.46±0.01 mg/mL, and 0.58±0.04 mg/mL, respectively.

Table 3. IC50 values (mg/mL), inhibition type, and Ki values (mg/mL) of *Vaccinium arctostaphylos* L. fruit extracts against α-glucosidase enzyme

Extracts	IC_{50} values	Inhibition type	K_i values
EE	0.301±0.003	Noncompetitive	0.48±0.02
ME	0.477±0.003	Noncompetitive	0.46±0.01
AE	0.591±0.007	Competitive	0.58±0.04
Acarbose	0.031±0.001	-	-

EE: Ethanol extract, ME: Methanol extract, AE: Aqueous extract

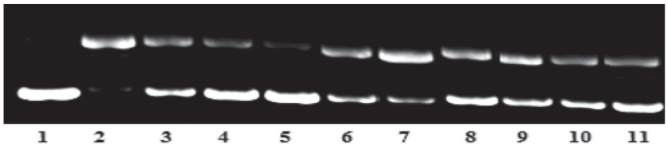

Figure 3. DNA protective properties of *Vaccinium arctostaphylos* L. fruit extracts. Lane 1: DNA control; Lane 2: DNA + 1 mM $FeSO_4$ + 2% H_2O_2; Lane 3: DNA + 1 mM $FeSO_4$ + 2% H_2O_2 + 0.125 mg/mL EE; Lane 4: DNA + 1 mM $FeSO_4$ + 2% H_2O_2 + 0.25 mg/mL EE; Lane 5: DNA + 1 mM $FeSO_4$ + 2% H_2O_2 + 0.5 mg/mL EE; Lane 6: DNA + 1 mM $FeSO_4$ + 2% H_2O_2 + 0.125 mg/mL ME; Lane 7: DNA + 1 mM $FeSO_4$ + 2% H_2O_2 + 0.25 mg/mL ME; Lane 8: DNA + 1 mM $FeSO_4$ + 2% H_2O_2 + 0.5 mg/mL ME; Lane 9: DNA + 1 mM $FeSO_4$ + 2% H_2O_2 + 0.125 mg/mL AE; Lane 10: DNA + 1 mM $FeSO_4$ + 2% H_2O_2 + 0.25 mg/mL AE; Lane 11: DNA + 1 mM $FeSO_4$ + 2% H_2O_2 + 0.5 mg/mL AE

AE: Aqueous extract, EE: Ethanol extract, ME: Methanol extract

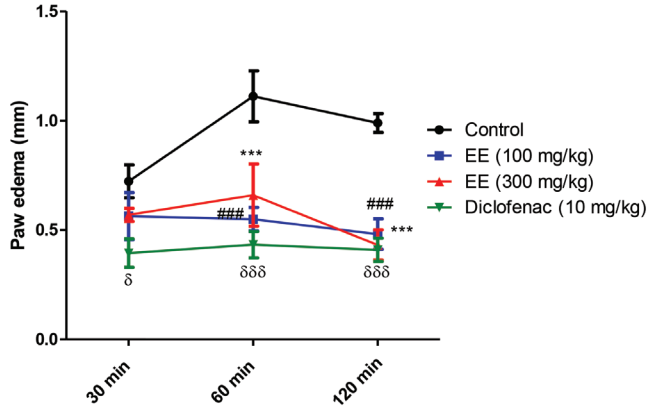

Figure 4. Effect of EE of *Vaccinium arctostaphylos* L. fruits in formalin-induced paw edema in mice (n=6)

###p<0.001 EE (100 mg/kg) vs control group, ***p<0.001 EE (300 mg/kg) vs control group, δp<0.05; δδδp<0.001 diclofenac (10 mg/kg) vs control group (two-way ANOVA, post-hoc Bonferroni), EE: Ethanol extract

In vivo anti-inflammatory activity

The *in vivo* anti-inflammatory activity of EE was also evaluated due to its higher antioxidant activity than the other extracts. As presented in Figure 3, the intraplantar injection of formalin solution induced edema in the control group significantly with a peak at 60 min. Pretreatment with 100 and 300 mg/kg doses of EE significantly reduced the edematogenic response at 60 and 120 min compared to the control group (p<0.001). As expected, diclofenac treatment markedly reduced edema thickness at 30, 60, and 120 min compared to the control group (p<0.05; p<0.001). However, there was no statistically significant difference between extract doses or extract doses and the diclofenac group in anti-edematogenic response.

DNA protective properties

The DNA protective properties of extracts were investigated using supercoiled pBR322 plasmid DNA against damage caused by hydroxyl (·OH) radicals and the results are shown in Figure 4. When supercoiled pBR322 plasmid DNA (form I) was exposed to Fenton's reagent ($FeSO_4$ and H_2O_2), form I converted to nicked pBR322 plasmid DNA (form II) by single-strand breaks as shown in lane 2 in Figure 4. Upon increasing concentration of the extracts treated with pBR322 DNA, form II decreased and form I increased in a concentration dependent manner. At 500 µg/mL, EE almost converted form II to form I; thereby it had the highest protective effect among the extracts.

DISCUSSION

The phenolic compounds, acting as hydrogen donors, ROS scavengers, and reducing agents, are responsible for many biological activities such as hepatoprotective, anti-allergic, anticancer, anti-inflammatory, antimutagenic, antioxidant, and antidiabetic effects.[35] In the present work, EE had the highest total phenolic content, with 44.42±1.22 mg GAE/g dry weight. According to the literature, Ayaz et al.[14] reported that 13 phenolic compounds were identified in *V. arctostaphylos* fruits from Turkey, including gallic, protocatechuic, *p*-hydroxybenzoic, *m*-hydroxybenzoic, gentisic, sinapic, chlorogenic, *p*-coumaric,

ferulic, syringic, caffeic, salicylic, and trans-cinnamic acids. Saral et al.[36] reported that total phenolic contents of ME in *V. arctostaphylos* fruits from different regions were 20.74±0.24 mg GAE/g weight of samples. Hasanloo et al. [37] reported that acidic ME of the plants was found to contain 9.48 mg GAE/g dry weight. The higher amount of total phenolic content was determined as 42.73 mg GAE/g dry weight in Iran and the highest phenolic content was determined in May. Anthocyanins, which are responsible for colors ranging from red to blue in most vegetables, flowers, and fruits, are water-soluble pigments that are extensively spread throughout the plant kingdom. These compounds have been reported to have anti-inflammatory and protective effects against chronic disorders such as hypertension, DM, and metabolic syndromes.[38] Latti et al.[15] identified that delphinidin, petunidin, malvidin were the most predominant anthocyanidins in *V. arctostaphylos* fruits from Turkey using high performance liquid chromatography (HPLC)-diode array detection and HPLC-electrospray ionization-mass spectrometer. In the present study, EE had the highest total anthocyanin content, with 8.46±0.49 mg CGE/g dry weight among the extracts tested. Similar to our findings, Saral et al.[36] reported that ME of *V. arctostaphyl*os was 6.14±0.01 mg CGE/g dry weight. The results obtained in the present study demonstrated that *V. arctostaphylos* is a rich source of secondary metabolites.

The flavonoid compounds, which are secondary metabolites, are crucial constituents due to their active hydroxyl groups.[39] In the present study, the results for total flavonoid were found to range from 9.22±0.92 mg QEE/g dry weight to 1.40±0.02 mg QE/g dry weight. According to the results of Mohaddese et al.'s[11] study, total flavonoid contents of AE, EE, and ME of *V. arctostaphylos* fruits were 5.4, 7.2, and 5.5 mg QEE/g dry weight, respectively, while Saral et al.[36] reported that ME of it ranged from 1.93±0.10 to 2.16±0.46 mg QEE/g dry weight. In the present work, we determined the antioxidant activities of EE, ME, and AE of *V. arctostaphyl*os fruits on the basis of DPPH and metal chelating, radical scavenging, and reducing power. DPPH, a stable nitrogen free radical, is generally used to determine the scavenging activities of compounds that eliminate this radical with electron donation or hydrogen atom transfer.[40] EE showed higher DPPH scavenging activity and was positively correlated with total phenolic content. The correlation of total phenolic, total anthocyanin, and total flavonoid contents with DPPH was determined using GraphPad Prism 5.0. The Pearson's correlation coefficient (r) and coefficient of determination (R^2) results for total phenolic, total anthocyanin, and total flavonoid contents with DPPH were r=0.996 and R^2=0.992, r=0.830 and R^2=0.689, and r=0.990 and R^2=0.980, respectively. In addition, there is a correlation between total anthocyanin and metal chelating effects with r=0.972 and R^2=0.945. Mohaddese et al.[11] reported that SC_{50} values of DPPH radical scavenging of AE, EE, and ME were 75, 45, and 35 µg/mL, respectively. In addition, Jooyandeh et al.[13] prepared ultrasound-assisted extract and reported that *V. arctostaphylos* fruits were scavenged at a rate of 32.21% at 1 mg/mL.The FRAP assay is an antioxidant method to determine the reducing capacity of samples *in vitro*. In the present study, the FRAP of extracts was demonstrated in the following order: EE>ME>AE. Güder et al.[12] reported that *V. arctostaphylos* fruits have remarkable reducing activities at different temperatures. The correlation between the FRAP with total anthocyanin and total phenolic was determined as r=0.950 and R^2=0.903 and r=0.933 and R^2=0.870.

There are many reports that suggest that phenolic, anthocyanin, and flavonoid compounds included in medicinal herbs are responsible for α-glucosidase inhibition.[41,42] According to these results, the α-glucosidase inhibitory effect with total phenolic and total anthocyanin contents is more compatible than that between the α-glucosidase inhibitory effect with total flavonoid content. Feshani et al.[43] reported that EE of *V. arctostaphylos* fruits showed antihyperglycemic activity against diabetic rats. The correlation between the α-glucosidase inhibitory effect with total phenolic, total anthocyanin, and total flavonoid contents was determined as r=0.993 and R^2=0.986, r=0.986 and R^2=0.972, and r=0.815 and R^2=0.665.

The results from the Lineweaver–Burk plots are presented in Table 3 and Figure 1. EE and ME inhibited α-glucosidase in a noncompetitive manner with K_i values of 0.48±0.02 mg/mL and 0.46±0.01 mg/mL, respectively. The noncompetitive inhibitors increase V_{max} values and do not change K_m values against enzymes. The noncompetitive inhibitors bind to different sites on the enzyme or enzyme-substrate complex, but do not bind to active sites. Otherwise, AE did not change the V_{max} value and decreased the K_m value and so it was a competitive inhibitor with K_i values of 0.58±0.04 mg/mL.

The formalin-induced paw edema test is widely used to screen new potential anti-inflammatory agents.[44] In the present work, we used this model to evaluate the anti-inflammatory effect of EE and we found a significant reduction in formalin-induced edema for both doses of EE at 60 and 120 min when compared with the control group. This result suggested that EE of *V. arctostaphylos* could have a significant effect on the prevention of inflammatory response. In addition, it is well known that especially free radicals play a major role in several inflammatory diseases. In the present study, we have shown that *V. arctostaphylos* extracts exhibited potent antioxidant activity due to the diversity of their chemical compounds such as anthocyanins, phenolics, and flavonoids.[45,46] The antioxidant activity of EE might be related to its anti-inflammatory activity.

It is well known that Fenton's reagent triggers oxidative damage to the bases of DNA via formation of hydroxyl radicals. Medicinal plants including antioxidants prevent hydroxyl radical-induced DNA damage due to their scavenging activities.[47] According to the literature, several phenolic and flavonoid compounds protect DNA against the toxic and mutagenic effects of H_2O_2.[48] In the present work, increasing concentrations of the extracts prevented the cleavage of supercoiled plasmid DNA when exposed to Fenton's reagent. All of the extracts in our study demonstrated remarkable reduction in the formation of form II and increase in the formation form I. EE was remarkably effective in protecting DNA by inhibiting form II and these results may be associated with its antioxidant activities.

CONCLUSIONS

This study presented the antioxidant, α-glucosidase inhibitory, anti-inflammatory, and DNA protective properties of *V. arctostaphylos* fruit extracts from Turkey. The study data demonstrated that EE had the highest total phenolic, anthocyanin, and flavonoid contents and exhibited significant scavenging and reducing activities compared to the other extracts. In addition, there was a correlation between antioxidant results and total phenolic, anthocyanin, and flavonoid contents. The α-glucosidase inhibitory studies revealed that EE and ME inhibited enzyme with IC_{50} values of 0.301±0.002 mg/mL and 0.477±0.003 mg/mL and were determined as noncompetitive inhibitors, while AE was a competitive inhibitor. The α-glucosidase inhibitory properties of extracts were in the following order: EE>ME>AE. In the anti-inflammatory experiment, EE indicated a significant reduction in formalin-induced edema in mice. In addition, when DNA was exposed to Fenton's reagent, all of extracts protected the DNA from damage, especially EE due to its antioxidant capacity. These results suggest that EE of *V. arctostaphylos* L. might be promising for the treatment or prevention of many diseases associated with oxidative damage and inflammation. Further studies are required to confirm these biological activities and mechanisms of action.

ACKNOWLEDGEMENTS

This work was supported by grants from Karadeniz Technical University. We are grateful to Professor Kamil Coşkunçelebi for his help with the authentication of the species.

Conflict of interest: There are no conflicts of interest among the authors.

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