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

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

ÖZ

Amaç: Bu çalışmamın amacı Vaccinium arctostaphylos L.'den hazırlanan etanol (EE), metanol (ME) ve su (AE) ekstraktlarının toplam fenolik, antociyanin, flavonoid içeriklerini ve biyolojik özelliklerinin incelemesidir.

Gereç ve Yöntemler: V. arctostaphylos'ın EE, ME, ve AE ekstraktları hazırlanmıştır. Bu ekstraktların toplam fenolik, antociyanin, flavonoid içerikleri, antiosyandı (2,2’-difenil-1-pikrilhidrazil ferrous ion-chelating, ve ferric reducing antioxidant power assays), α-glucosidaz inhibitory, anti-inflamatuvar, ve DNA koruyucu 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, antociyanin, ve flavonoid içeriğine sahip olduğu görülmüştür. Bu ekstraktların antioksidan aktiviteleri sırasıyla EE>ME>AE olduğu belirlendi. EE ve ME α-glucosidaz inhibitory ve DNA koruyucu etkileri artmış, ancak AE ekstraktının inhibitory etkileri olmamıştır. Bromelin ve propolis ekstraktlarının inhibitory etkileri EE ve ME ekstraktlarının inhibitory etkilerine göre daha fazla olduğu tespit edilmiştir.

Sonuç: Bu çalışma, Vaccinium arctostaphylos L.'nin EE ve ME ekstraktlarının antiosyandı, anti-inflamatuar, ve DNA koruyucu etkilerinin belirlenmesi için bir ajans oluşturmuş olabilir.

Anahtar kelimeler: Antioksidan, anti-inflamatuar, DNA, α-glucosidaz, Vaccinium arctostaphylos

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 IC50 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) 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

*Correspondence: E-mail: burakbarut@ktu.edu.tr, Phone: +90 537 592 44 89 ORCID ID: orcid.org/0000-0002-7441-8771
Received: 14.02.2018, Accepted: 15.03.2018

©Turk J Pharm Sci, Published by Galenos Publishing House.
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. Many plants are considered able to scavenge and hinder free radicals, including reactive oxygen species (ROS) such as hydroxyl radical (\(\text{OH}^\cdot\)), hydrogen peroxide (H\(_2\)O\(_2\)), and superoxide anion radical (O\(_2^\cdot\)), which induce oxidative damage in biomolecules due to these secondary metabolites possessing antioxidant activity. In addition, plant-based natural antioxidants are preferred to synthetic ones due to their good safety profiles. 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. Numerous studies have reported that Vaccinium possesses several biological and pharmacological activities, making it an attractive medical plant. Previous studies reported that Vaccinium species have been used for memory improvement, eyesight protection, cardiovascular protection, and for their antioxidant, anti-diabetic, and anticancer activities.

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. 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. 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.

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. 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 people are living with DM; this number is expected to increase by 2045 approximately. In addition, 352 million people are living with DM; this number is expected to increase by 2045 approximately.

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.

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.

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.

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.

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. \(A_{\text{control}}\) is the antioxidant activity without extracts and \(A_{\text{extract}}\) is the antioxidant activity with extracts at various concentrations. \(S_{\text{50}}\) 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 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 (BHAEE) per g dry weight of the sample.
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:

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

Here $A_{\text{control}}$ is the activity of enzyme without extract and $A_{\text{extract}}$ 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. The kinetic analysis was conducted with various 4-pNP concentrations in the absence and presence of extracts.

**DNA protective properties**

The DNA protective properties of extracts of *V. arctostaphylos* fruits against oxidative damage caused by OH· were monitored by conversion of supercoiled plasmid pBR322 DNA to open circular form as described by Yeung et al. 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$_2$O$_2$, 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.

**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.

**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.

**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 radicals 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**

| Extract  | 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                                     | 4.0±0.02                                    |

| Extract  | SC$_{50}$ values (mg/mL) | Metal chelating effect (IC$_{50}$ values mg/mL) | FRAP (mg BHAE/g dry weight) |
|----------|--------------------------|-----------------------------------------------|-----------------------------|
| EE       | 0.141±0.009              | 0.45±0.007                                    | 62.06±2.13                  |
| ME       | 0.211±0.011              | 0.757±0.004                                   | 47.7±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 equivalents, CGE: Cyaniding-3-glucoside equivalents, QEE: Quercetin 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 BHAЕ/g dry weight. EE had the highest reducing activities, with 62.06±2.13 mg BHAЕ/g dry weight, while ME and AE were 47.70±2.77 and 15.39±0.98 mg BHAЕ/g dry weight, respectively.

**Enzyme inhibition and kinetic analysis of \( \alpha \)-glucosidase inhibition**

The \( \alpha \)-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 \( \alpha \)-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 \( \alpha \)-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. IC\( _{50} \) values (mg/mL), inhibition type, and \( K_i \) values (mg/mL) of Vaccinium arctostaphylos L. fruit extracts against \( \alpha \)-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    | 0.59±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 Fe\( _{2} \)O\( _{3} \) + 2% H\( _{2} \)O\( _{2} \); Lane 3: DNA + 1 mM Fe\( _{2} \)O\( _{3} \) + 2% H\( _{2} \)O\( _{2} \) + 0.125 mg/mL EE; Lane 4: DNA + 1 mM Fe\( _{2} \)O\( _{3} \) + 2% H\( _{2} \)O\( _{2} \) + 0.25 mg/mL EE; Lane 5: DNA + 1 mM Fe\( _{2} \)O\( _{3} \) + 2% H\( _{2} \)O\( _{2} \) + 0.5 mg/mL EE; Lane 6: DNA + 1 mM Fe\( _{2} \)O\( _{3} \) + 2% H\( _{2} \)O\( _{2} \) + 0.125 mg/mL ME; Lane 7: DNA + 1 mM Fe\( _{2} \)O\( _{3} \) + 2% H\( _{2} \)O\( _{2} \) + 0.25 mg/mL ME; Lane 8: DNA + 1 mM Fe\( _{2} \)O\( _{3} \) + 2% H\( _{2} \)O\( _{2} \) + 0.5 mg/mL ME; Lane 9: DNA + 1 mM Fe\( _{2} \)O\( _{3} \) + 2% H\( _{2} \)O\( _{2} \) + 0.125 mg/mL AE; Lane 10: DNA + 1 mM Fe\( _{2} \)O\( _{3} \) + 2% H\( _{2} \)O\( _{2} \) + 0.25 mg/mL AE; Lane 11: DNA + 1 mM Fe\( _{2} \)O\( _{3} \) + 2% H\( _{2} \)O\( _{2} \) + 0.5 mg/mL AE

**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, *p<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 (Fe\( _{2} \)O\( _{3} \) and H\( _{2} \)O\( _{2} \)), 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 \( \mu \)g/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. 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.\(^\text{14}\) 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, and other compounds.

According to the literature, Ayaz et al.\(^\text{14}\) 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. reported that total phenolic contents of ME in V. arctostaphylos fruits from different regions were 20.74±0.24 mg GAE/g dry weight of samples. Hasanloo et al. 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. Latti et al. 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. reported that ME of V. arctostaphylos 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. In the present study, the results for total flavonoid were found to range from 9.22±0.92 mg QE/g dry weight to 1.40±0.02 mg QE/g dry weight. According to the results of Mohaddese et al.’s study, total flavonoid contents of AE, EE, and ME of V. arctostaphylos fruits were 5.4, 7.2, and 5.5 mg QE/g dry weight, respectively, while Saral et al. reported that ME of it ranged from 1.93±0.10 to 2.16±0.46 mg QE/g dry weight. In the present work, we determined the antioxidant activities of EE, ME, and AE of V. arctostaphylos 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. 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 (R²) results for total phenolic, total anthocyanin, and total flavonoid contents with DPPH were r=0.996 and R²=0.992, r=0.830 and R²=0.689, and r=0.990 and R²=0.980, respectively. In addition, there is a correlation between total anthocyanin and metal chelating effects with r=0.972 and R²=0.945. Mohaddese et al. reported that SC values of DPPH radical scavenging of AE, EE, and ME were 75, 45, and 35 μg/mL, respectively. In addition, Jooyandeh et al.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. 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 R²=0.903 and r=0.933 and R²=0.870. There are many reports that suggest that phenolic, anthocyanin, and flavonoid compounds included in medicinal herbs are responsible for α-glucosidase inhibition. 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. reported that EE of V. arctostaphylos fruits showed anti-hyperglycemic 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 R²=0.986, r=0.986 and R²=0.972, and r=0.815 and R²=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 values of 0.48±0.02 mg/mL and 0.46±0.01 mg/mL, respectively. The noncompetitive inhibitors increase Vmax values and do not change Km 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 Vmax value and decreased the Km value and so it was a competitive inhibitor with Km values of 0.58±0.04 mg/mL.

The formalin-induced paw edema test is widely used to screen new potential anti-inflammatory agents. In the present work, we used this model to evaluate the anti-inflammatory effect of EE and 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. 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. According to the literature, several phenolic and flavonoid compounds protect DNA against the toxic and mutagenic effects of H2O2. 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 IC₅₀ 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.

REFERENCES

1. Seebaluck-Sandoram, R, Lall, N, Fibrich, B, Bloom van Staden, A, Mahomoodally, F. Antibiotic-potention, antioxidant, cytotoxic, anti-inflammatory and anti-acetylcholinesterase potential of *Antidesma madagascariense* Lam. (Euphorbiaceae). S Afr J Bot. 2017;111:194-201.

2. Supasuteekul, C, Nonhitipong, W, Tadtong, S, Likhitwitayawuid, K, Tengannuy, P, Sritularak, B, Antioxidant, DNA damage protective, neuroprotective, and α-glucosidase inhibitory activities of flavonoid glycoside from leaves of *Garcinia gracilis*. Rev Bras Farmacog. 2016;26:312-320.

3. Hyun, T, Kim, H, Ko, YJ, Kim, JS. Antioxidant, α-glucosidase inhibitory and anti-inflammatory effects of aerial parts extract from Korean crowberry (*Emeptrum nigrum* var. japonicum). Saudi J Biol Sci. 2016;23:181-188.

4. Feng, C, Wang, WW, Ye, JF, Li, SS, Wu, Q, Yin, DD, Li, B, Xu, YJ, Wang, LS. Polyphenol profile and antioxidant activity of the fruit and leaf of *Vaccinium glaucomus* from the Tibetan Himalayas. Food Chem. 2017;219:490-495.

5. Ahmadi, A, Khalili, M, Mashae F, Nahriz-Niknafs, B. The effects of solvent polarity on hypoglycemic and hypolipidemic activities of *Vaccinium arctostaphylos* L. Unripe fruits. Pharm Chem J. 2017;50:746-752.

6. Krajalyte, V, Venskutonis, PR, Pukalskas, A, Cesoniene, L, Daubaras, R. Antioxidant properties, phenolic composition and potentiometric sensor array evaluation of commercial and new blueberry (*Vaccinium corymbosum*) and bog blueberry (*Vaccinium uliginosum*) genotypes. Food Chem. 2015;188:583-590.

7. Cambers, BK, Camire, ME. Can cranberry supplementation benefit adults with type 2 diabetes. Diabetes Care. 2003;26:2695-2696.

8. Kraft, TFB, Schmidt, BM, Yosuf, GG, Knight, CTG, Cundert, M, Kang, YH, Pezzuto, JM, Siegel, DS, Lila, MA. Chemopreventive potential of wild lowbush blueberry fruits in multiple stages of carcinogenesis. J Food Sci. 2005;70:159-166.

9. Krikorian, R, Shidler, MD, Nash, TA, Kalt, V, Vinqvist-Tymchuk, MR, Shukitt-Hale, B, Joseph, JA. Blueberry supplementation improves memory in older adults. J Agric Food Chem. 2010;58:3996-4000.

10. Liu, Y, Song, X, Han, Y, Zhou, F, Zhang, D, Ji, B, Hu, J, Lv, Y, Cai, S, Wei, Y, Gao, F, Jia, X. Identification of anthocyanin components of wild Chinese blueberries and amelioration of light induced retinal damage in pigmented rabbit using whole berries. J Agric Food Chem. 2011;59:356-363.

11. Mohadese, M, Kazempour, N, Taghizadeh, M. *In vitro* antimicrobial and antioxidant activity of *Vaccinium arctostaphylos* L. extracts. Journal of Biologically Active Products from Nature. 2013;3:241-247.

12. Güder, A, Engin, MS, Yolcu, M, Gür, M. Effect of processing temperature on the chemical composition and antioxidant activity of *Vaccinium arctostaphylos* fruit and their jam. J Food Process Preserv. 2014;38:1696-1704.

13. Jooyandeh, H, Noshad, M, Khamirian, R, Modeling of ultrasound-assisted extraction, characterization and *in vitro* pharmacological potential of polysaccharides from Vaccinium arctostaphylos L. Int J Biol Macromol. 2018;107:938-948.

14. Ayaz, FA, Hayriloğlu, Ayaz, S, Gruz, J, Novak, O, Strnad, M. Separation, characterization, and quantitation of phenolic acids in a little-known blueberry (*Vaccinium arctostaphylos* L.) fruit by HPLC-MS. J Agric Food Chem. 2005;53:8116-8122.

15. Latti, AK, Kainulainen, PS, Hayriloğlu-Ayaz, S, Ayaz, FA, Rihinen, KR. Characterization of anthocyanins in caucasian blueberries (Vaccinium arctostaphylos L.) native to Turkey. J Agric Food Chem. 2009;57:5244-5249.

16. Deliorman, Orhan, D, Orhan, N. Assessment of *In Vitro* Antidiabetic and Antioxidant Effects of *Helianthus tuberosus*, *Cynodia oblonga* and *Allium porrum*. Turk J Pharm Sci. 2016;13:181-188.

17. Şöhretoğlu, D, Sari, S, Soral, M, Barut, B, Özel, A, Liptaj, T. Potential of *Potentilla inclinata* and its polyphenolic compounds in α-glucosidase inhibition: Kinetics and interaction mechanism merged with docking simulations. Int J Biol Macromol. 2018;108:81-87.

18. International Diabetes Federation. Diabetes Atlas. www.idf.org/ diabetesatlas (Accessed 9 March 2018) 2017.

19. Sulistiyani, Safithri, M, Sari, YP. Inhibition of α-glucosidase activity by ethanolic extract of *Melia azedarach* L. leaves. IOP Conf Ser Earth Environ Sci. 2016;70:1-5.

20. Zhang, J, Zhao, S, Yin, P, Yan, L, Han, J, Shi, L, Zhou, X, Liu, Y, Ma, C. α-Glucosidase Inhibitory Activity of Polyphenols from the Burs of *Castanea mollissima* Blume. Molecules. 2014;19:8373-8386.

21. Barut, EN, Barut, B, Engin, S, Yildirim, S, Yaşar, A, Türkş, S, Özel, A, Sezen, FS. Antioxidant capacity, anti-acetylcholinesterase activity and inhibitory effect on lipid peroxidation in mice brain homogenate of *Achillea millefolium*. Turk J Biochem. 2017;42:493-502.

22. Keser, S, Çelik, S, Türkşöglü, S, Yılmaz, O, Türkşöglü, İ. Antioxidant activity, total phenolic and flavonoid content of water and ethanol extracts from *Achillea millefolium* L. Turk J Pharm Sci. 2013;10:385-392.
23. Cheng GW, Breen PJ. Activity of phenylalanine ammonialyase (PAL) and concentrations of anthocyanins and phenolics in developing strawberry fruit. J Am Soc Hortic Sci. 1991;116:865-869.

24. Chang CC, Yang MH, Wen HM, Chern JC. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J Food Drug Anal. 2002;10:178-182.

25. Bakar F, Bahadır Ackkara Ö, Ergene B, Nebioğlu S, Saltan Çitoğlu G. Antioxidant activity and phytochemical screening of some Asteraceae Plants. Turk J Pharm Sci. 2015;12:123-132.

26. Chua MT, Tung YT, Chang ST. Antioxidant activities of ethanolic extracts from the twigs of Cinnamomum osmophleum. Bioresour Technol. 2008;99:1918-1925.

27. Oyaizu M. Studies on products of browning reactions-antioxidative activities of products of browning reaction prepared from glucosamine. Jpn J Nutr. 1986;44:307-315.

28. da Silva Pinto M, Kwon YI, Apostolidis E, Lajolo FM, Genovese MI, Shetty K. Functionality of bioactive compounds in brazilian strawberry (Fragaria × Ananassa Duch.) cultivars: evaluation of hyperglycemia and hypertension potential using in vitro models. J Agric Food Chem. 2008;56:4386-4392.

29. Lineweaver H, Burk D. The determination of enzyme dissociation constant. J Am Chem Soc. 1934;56:658-666.

30. Şöhretoglu D, Sari S, Özel A, Barut B. α-Glucosidase inhibitory effect of Potentilla astracanica and some isoflavones: inhibition kinetics and mechanistic insights through in vitro and in silico studies. Int J Biol Macromol. 2017;105:1062-1070.

31. Yeung SY, Lan WH, Huang CS, Lin CP, Chan CP, Chang MC, Jeng JH. Scavenging property of three cresol isomers against H2O2, hypochlorite, superoxide and hydroxyl radicals. Food Chem Toxicol. 2002;40:1403-1413.

32. Barut B, Demirbaş Ü, Özel A, Kantekin H. Novel water soluble morpholine substituted Zn(II) phthalocyanine: Synthesis, characterization, DNA/BSA binding, DNA photocleaveage and topoisomerase I inhibition. Int J Biol Macromol. 2017;105:499-508.

33. Kumar T, Jain V. Antinociceptive and anti-inflammatory activities of Bridelia retusa methanolic fruit extract in experimental animals. Scientific World Journal. 2014;2014:890151.

34. Kumar S, Sandhir R, Ojha S. Evaluation of antioxidant activity and total phenol in different varieties of Lantana camara leaves. BMC Res Notes. 2014;7:560.

35. Alam MA, Zaidul IS, Ghafoor K, Sahena F, Hakim MA, Rafii MY, Abir HM, Bostanudin MF, Perumal V, Khatib A. In vitro antioxidant and α-glucosidase inhibitory activities and comprehensive metabolite profiling of methanol extract and its fractions from Clinacanthus nutans. BMC Complement Altern Med. 2017;17:181.

36. Saral Ö, Öğuz Z, Şahin H. Comparison of Antioxidant Properties of Wild Blueberries (Vaccinium arctostaphylos L. and Vaccinium myrtillus L.) with Cultivated Blueberry Varieties (Vaccinium corymbosum L.) in Artvin Region of Turkey. Turk J Ag Food Sci Techn. 2015;3:40-44.

37. Hasanloo T, Sepehrifar R, Hajimehdipoor H. Levels of phenolic compounds and their effects on antioxidant capacity of wild Vaccinium arctostaphylos L. (Qare-Qat) collected from different regions of Iran. Turk J Biol. 2011;35:371-377.

38. Yıldırım S, Kadioğlu A, Sağlam A, Yaşar A, Selilitpe HE. Fast determination of anthocyanins and free pelargonidin in fruits, fruit juices, and fruit wines by high-performance liquid chromatography using a core-shell column. J Sep Sci. 2016;39:3927-3935.

39. Raffa D, Maggio B, Raimondi MV, Plescia F, Daidone G. Recent discoveries of anticancer flavonoids. Eur J Med Chem. 2017;142:213-228.

40. Kazeem MI, Ashafa AOT. In vitro antioxidant and antidiabetic potentials of Dianthus basalicus Burtt Davy whole plant extracts. J Herb Med. 2015;5:158-164.

41. Jimenez-Suarez V, Nieto-Camacho A, Jimenez-Estrada M, Alvarado Sanchez B. Anti-inflammatory, free radical scavenging and alpha-glucosidase inhibitory activities of Hamelia patens and its chemical constituents. Pharm Biol. 2016;54:1822-1830.

42. Zlotek U, Szchowski KA, Swieca M. Potential in vitro antioxidant, anti-inflammatory, antidiabetic, and anticancer effect of arachidonic acid-elicted basil leaves. J Funct Foods. 2017;36:290-299.

43. Feshani AM, Kouhsari SM, Mohammadi S. Vaccinium arctostaphylos, a common herbal medicine in Iran: Molecular and biochemical study of its antidiabetic effects on alloxan-diabetic Wistar rats. J Ethnopharmacol. 2011;133:67-74.

44. Mohammad FE, Hasan WA, Mohamed EG. Natural antioxidant flavonoids in formalin-induced mice paw inflammation; inhibition of mitochondrial sorbitol dehydrogenase activity. J Biochem Mol Toxicol. 2017;31:21896.

45. Bowen-Forbes CS, Zhang Y, Nair MG. Anthocyanin content, antioxidant, and anti-diabetic effects on alloxan-diabetic rat. Food Chem. 2011;128:109-116.