Investigation of antioxidant, α-glucosidase inhibitory, anti-inflammatory and DNA protective properties of *Vaccinium arctostaphylos* L.

Short title: Biological activities of *V. arctostaphylos* L.

ABSTRACT

Objective: The scope of this study was to investigate total phenolic, anthocyanin, flavonoid contents and biological properties of ethanol extract (EE), methanol extract (ME) and aqueous extract (AE) from *Vaccinium arctostaphylos* (*V. 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, antioxidant (DPPH, ferrous ion-chelating and FRAP 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 GAE/g dry weight, 8.46 ± 0.49 mg/CGE/g dry weight and 9.22 ± 0.92 mg QEE/g dry weight, respectively. The antioxidant activities of extracts followed 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 inhibitor with the inhibitory constant (K_i) values of 0.48 ± 0.02 mg/mL and 0.46 ± 0.01 mg/mL. 100 and 300 mg/kg doses of EE caused a significant reduction in formalin-induced edema in mice, demonstrating anti-inflammatory effect of EE. In DNA protective studies, all of extracts prevented 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 effective medical plant to prevent or treat diseases associated with oxidative damage and inflammation.

Keywords: antioxidant, anti-inflammatory, DNA, α-glucosidase, *V. arctostaphylos*.

***Vaccinium arctostaphylos* L.'nin antioksidan, α-glukozidaz inhibisyon, anti-inflamatuvar ve DNA koruma özelliklerinin incelenmesi**

ÖZ

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

Materyal ve Metods: *V. arctostaphylos*'un EE, ME ve AE ekstraktları hazırlandı. Bu ekstraktların total fenolik, antosiyanin ve flavonoid içerikleri, antioksidan (DPPH, metal iyon şelatlama ve FRAP metotları), α-glukozidaz, anti-inflamatuvar ve DNA koruma özellikleri araştırılmıştır.

Bulgular: EE, 44.42 ± 1.22 mg GAE/g kuru ağırlık, 8.46 ± 0.49 mg/CGE/g kuru ağırlık ve 9.22 ± 0.92 mg QEE/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-inflamatuar, 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, *V. arctostaphylos.*

INTRODUCTION

Medicinal plants, contain secondary metabolites such as phenolic, anthocyanin, flavonoid compounds, have been used as alternative therapeutic tools to treat many diseases throughout medical history (1). Many plants are considered to be able to scavenge and hinder the free radicals including the reactive oxygen species (ROS) such as hydroxyl radical ($OH^{\cdot}$), hydrogen peroxide (H_2O_2) and superoxide anion radical ($O2^{\cdot-}$) which induce oxidative damage in biomolecules due to these secondary metabolites possessing antioxidant activity (2). In addition, plant-based natural antioxidants are preferred to synthetics due to their good safety profiles (3). Therefore, there is a growing interest to find natural compounds that could prevent oxidative damage underlying the pathogenesis of many diseases.

The *Vaccinium* genus, belongs to the *Ericaceae* family, includes approximately 450 species and distributed in Northern Hemisphere and tropical mountains of America and Asia (4,5). Numerous studies 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, antioxidant, anti-diabetic and anticancer activities (7-10).

Vaccinium arctostaphylos (V. arctostaphylos) L., commonly named Caucasian whortleberry, is the only member of the *Vaccinium* genus and is widely used as anti-diabetic and anti-hypertensive agents (11,12). To date, this plant has been reported to contain phenolic compounds such as anthocyanin, flavanol, 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 found as 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 the insulin resistance (16,17). According to 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 glycosidic bond in oligosaccharides into α-glucose, resulting in postprandial hyperglycemia (19). Thus, 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, flatulence etc. (20). Therefore, scientists seek novel natural α-glucosidase inhibitors against DM.

To our knowledge, there is no report on the kinetic studies of α-glucosidase inhibition, anti-inflammatory and DNA protective properties of *V. arctostaphylos*. The goal of the present study was to evaluate 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 two weeks and the dried samples were pulverized using an automatic herbal grinder. Then, pulverized fruits were extracted with solvent (ethanol, methanol and water) in the shaker for 6 h × 3. After shaking, mixtures were filtered with Whatman filter paper No:1. The solvent was evaporated under reduced pressure by a Hei-Vap Heidolph 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 described method by Keser. The calibration curve was obtained with 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 method 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

DPPH radical scavenging assay

The DPPH radical scavenging activities of extracts were investigated using the method described by Blois and the inhibition percentage was calculated using Formula 1 (25).

Formula 1.

$A_{control}$ is the antioxidant activities without extracts and $A_{extract}$ is the antioxidant activities 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 method and the ferrous ion chelating capacities were calculated using Formula 1 (26).

Ferric reducing antioxidant power (FRAP) assay

The 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).

Enzyme inhibition

α-Glucosidase inhibition assay

The α-glucosidase inhibitory properties were examined according to previous study with a slight modificiation (28). In this study, the extracts and 0.5 U/mL α-glucosidase enzyme were mixed in 96-well microplate and waited to react for 10 min. After then, 5 mM 4-pNPG was added and the reaction mixture was incubated for 10 min. The absorbance was measured at 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:

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

Where $A_{control}$ 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 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 by 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 formed by hydroxyl radical were monitored by the conversion of supercoiled plasmid

pBR322 DNA to open circular form as described by Yeung et al. (31). In this study, the total volume of mixture 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 ºC for 1 h. After incubation, loading buffer (bromophenol, glycerol, SDS and xylene cyanol) was added the mixture. The mixtures were loaded on agarose gel and electrophoresis was performed at 100 V for 90 min using wide-midi-sub cell GT system BioRad. The results visualized with BioRad Gel Doc XR system (32).

In vivo anti-inflammatory activity

Animals

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 a period of one week. Experiments were carried out between 9 am and 4 pm. The experimental protocol was approved by the Institutional Animal Ethical Committee from Karadeniz Technical University (2017/45).

Formalin-induced hind paw edema

The anti-inflammatory activity of EE was evaluated by using formalin-induced edema method. Mice were divided into the following 4 groups of 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 mice for three consecutive days. 60 min after the last dose of extracts and 30 min after administration of diclofenac and saline, 20 µL 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 before 30 min and 30, 60, 120 min after the formalin injection by using micrometer caliper respectively (33).

Statistical analysis

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

RESULTS

Determination of total phenolic, anthocyanin and flavonoid contents

The results of total phenolic, total anthocyanin and total flavonoid contents of extracts were 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.

Evaluation of antioxidant activity

The SC_{50} values of DPPH and metal chelating radical scavenging activities of extracts were presented in Table 2. All extracts demonstrated scavenging activities against DPPH radical in a concentration-dependent manner. DPPH radical scavenging assay showed that EE had significant antioxidant activities with SC_{50} value of 0.141 ± 0.009 mg/mL. The extracts demonstrated moderate metal chelating activities compared to EDTA. EE had the highest chelating activities with SC_{50} value of 0.453 ± 0.007 mg/mL whereas AE had the lowest activities with SC_{50} value of 0.909 ± 0.006 mg/mL.

The FRAP activities of the extracts were 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, whilst ME and AE were 47.70 ± 2.77 and 15.39 ± 0.98 mg BHAE/ g dry weight.

Enzyme inhibiton and kinetic analysis of α-glucosidase inhibition

The α-glucosidase inhibitory effects of extracts were evaluated using da Silva Pinto method when compared to acarbose as a standard reference. The results obtained in this study were expressed as IC_{50} values and presented in Table 3. The extracts demonstrated inhibitory effect against α-glucosidase ranged 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 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 presented in Table 3, Figs 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 found to be 0.48 ± 0.02 mg/mL, 0.46 ± 0.01 mg/mL and 0.58 ± 0.04 mg/mL, respectively.

In vivo anti-inflammatory activity

In this study, *in vivo* anti-inflammatory activity of EE was also evaluated due to its higher antioxidant activity than the other extracts. As presented in Figure 4, the intraplantar injection

of formalin solution induced edema in 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 control group ($p < 0.001$). As expected diclofenac treatment markedly reduced edema thickness at the 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 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 shown in Figure 3. In this study, when supercoiled pBR322 plasmid DNA (Form I) were 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 shown in Lane 2 on Figure 3. Upon increasing concentration of the extracts were 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 other extracts.

DISCUSSION

The phenolic compounds, act as a hydrogen donor, ROS scavengers and reducing agents, are responsible for the many biological activities such as hepatoprotective, anti-allergic, anti-cancer, anti-inflammatory anti-mutagenic, antioxidant, and anti-diabetic effects etc. (35). In this work, EE had the highest total phenolic content with 44.42 ± 1.22 mg GAE/ g dry weight. According to the literature, Ayaz reported that thirteen 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 (14). Saral reported that total phenolic contents of ME of *V. arctostaphylos* fruits from different regions were found to range from 20.74 ± 0.24 mg GAE/ g weight of samples (36). Hasanloo 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 determined in May (37).

Anthocyanins, which are liable for colours ranging from red to blue of 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, diabetes mellitus and metabolic syndromes (38). Latti et al. identified that delphinidin, petunidin, malvidin were the

most predominant anthocyanidins in *V. arctostaphylo*s fruits from Turkey using HPLC-DAD and HPLC-ESI-MS (15). In this study, EE had the highest total anthocyanin content with 8.46 ± 0.49 mg CGE/g dry weight among extracts tested. Similar to our findings, Saral reported that ME of *V. arctostaphylo*s was 6.14 ± 0.01 mg CGE/g dry weight (36). The results obtained in this study demonstrated that *V. arctostaphylo*s is rich source of secondary metabolites.

The flavonoid compounds, one of the secondary metabolites, are crucial constituents due to the active hydroxyl groups (39). In this study, the results of 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 results of Mahboubi 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, Saral reported that ME of it was found to range from 1.93 ± 0.10 to 2.16 ± 0.46 mg QEE/g dry weight (11,37).

In this work, we determined the antioxidant activities of EE, ME and AE of *V. arctostaphylo*s fruits on the basis of DPPH and metal chelating radical scavenging, reducing power. DPPH, stable nitrogen free radical, is generally used to determine the scavenging activities of compounds which eliminate this radical with electron donation or hydrogen atom transfer (40). EE showed higher DPPH scavenging activities and 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 of total phenolic, total anthocyanin and total flavonoid contents with DPPH were r = 0.996, R^2 = 0.992; r = 0.830, R^2 = 0.689 and r = 0.990, R^2 = 0.980, respectively. In addition, there is a correlation between total anthocyanin and metal chelating effects with r= 0.972, R^2 = 0.945. According to literature, Mahboubi reported that SC_{50} values of DPPH radical scavenging of AE, EE and ME were determined as 75, 45 and 35 µg/mL, respectively (11). In addition, Jooyandeh prepared ultrasound-assisted extract and reported that *V. arctostaphylos* fruits were scavenged with 32.21% at 1 mg/mL (13).

The FRAP assay is one of the antioxidant methods to determine reducing capacity of the samples *in vitro*. In this study, the FRAP of extracts demonstrated in the following order: EE > ME > AE. According to literature, Güder reported that *V. arctostaphylos* fruits have remarkable reducing activities at different temperatures (12). The correlation between the FRAP with total anthocyanin and total phenolic were determined as r = 0.950, R^2 = 0.903; r = 0.933, R^2 = 0.870.

There are many reports suggest that phenolic, anthocyanin and flavonoid compounds included in medicinal herbs are responsible for α-glucosidase inhibitory effect (41,42).

According to these results, the α-glucosidase inhibitory effect with total phenolic, total anthocyanin contents have more compatible than between the α-glucosidase inhibitory effect with total flavonoid content. Feshani reported that EE of *V. arctostaphylos* fruits showed antihyperglycemic activity against diabetic rats (43). The correlation between the α-glucosidase inhibitory effect with total phenolic, total anthocyanin and total flavonoid contents were determined as r = 0.993, R^2 = 0.986; r = 0.986, R^2 = 0.972; r = 0.815, R^2 = 0.665.

The results from Lineweaver-Burk plots were presented in Table 3 and Figure 1. EE and ME inhibited α-glucosidase via 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 unchange K_m values against enzymes. The noncompetitive inhibitors bind to different sites on the enzyme or enzyme-substrate complex, not bind to active sites. Otherwise, AE unchanged V_{max} value and decreased K_m value so it was a competitive inhibitor with K_i values 0.58 ± 0.04 mg/mL.

Formalin-induced paw edema test is widely used to screen new potential anti-inflammatory agents (44). In this work, we used this model to evaluate anti-inflammatory effect of EE and we have found a significant reduction in formalin-induced edema for both doses of EE at 60 and 120 min when compared with control group. This result suggested that EE of *V. arctostaphylos* could have 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 reagents trigger oxidative damage bases of DNA via formation of hydroxyl radicals. The medicinal plants including antioxidants protect hydroxyl radical induced DNA damage due to their scavenging activities (47). According to literature, several phenolic and flavonoid compounds prevent DNA against toxic and mutagenic effects of H_2O_2 (48). In this work, increasing concentrations of the extracts prevented the cleavage of supercoiled plasmid DNA when exposed to Fenton's reagent. The all of extracts in this study demonstrated remarkable reduction in the formation of Form II and increasing in the formation Form I. EE was remarkably effective in protecting DNA by inhibiting the Form II and these results may be associated with its antioxidant activities.

CONCLUSION

This study presented antioxidant, α-glucosidase inhibitory, anti-inflammatory, and DNA protective properties of *V. arctostaphylos* fruits 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 among 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 whilst AE was a competitive inhibitor. The α-glucosidase inhibitory properties of extracts exhibited in the following order: EE > ME > AE. In anti-inflammatory experiment, EE indicated a significant reduction in formalin-induced edema in mice. In addition, when DNA were exposed to Fenton's reagent, all of extracts protected the DNA damage especially EE due to their antioxidant capacity. These results suggest that EE of *V. arctostaphylos* L. might be a promising medical plant for treatment or prevention of many diseases associated with oxidative damage and inflammation. Further studies are required to confirm these biological activities and mechanisms of actions.

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Table 1. Total phenolic, anthocyanin and flavonoid contents of *V. arctostaphylos* 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

Table 2. DPPH radical scavenging, metal chelating and FRAP activities of *V. Arctostaphylos* 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	-

Table 3. IC_{50} values (mg/mL), inhibition type and K_i values (mg/mL) of *V. arctostaphylos* 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	-	-

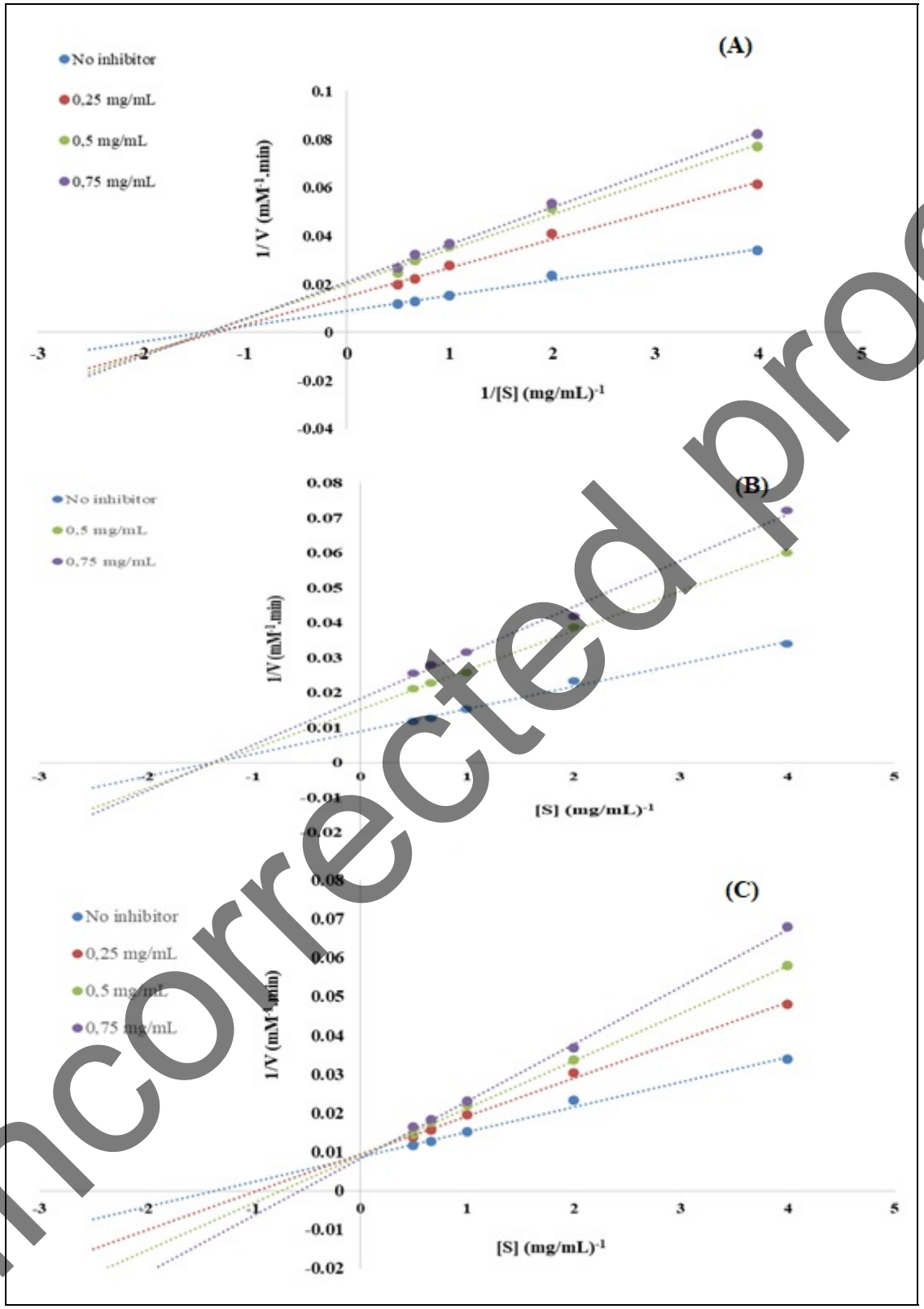

Figure 1. Lineweaver-Burk plots for kinetic analysis of α-glucosidase inhibition by EE (A), ME (B) and AE (C).

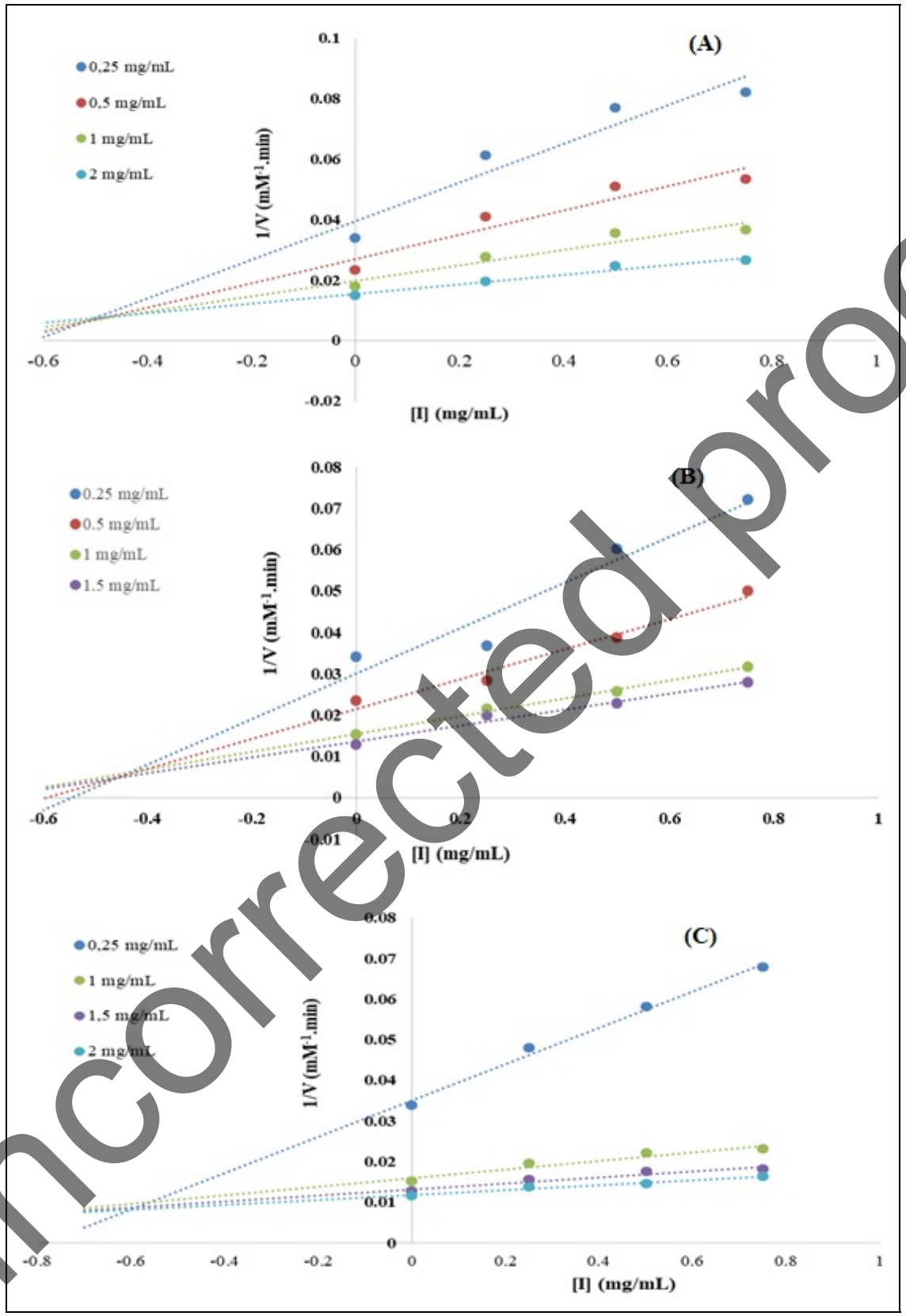

Figure 2. Dixon plot kinetic analysis of α-glucosidase inhibition by EE (A), ME (B) and AE (C).

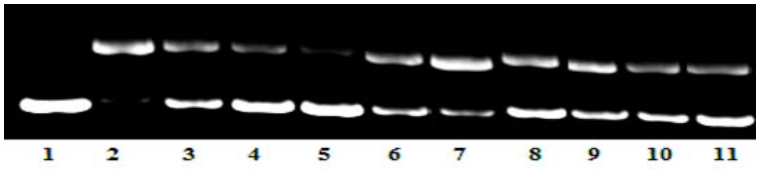

Figure 3. DNA protective properties of *V. arctostaphylos* fruit extracts. Lane 1: DNA control; Lane 2: DNA + 1 mM $FeSO_4$ + 2% H_2O_2; Lane 3: DNA + 1 mM $FeSO_4$ + 2% H_2O_2 + 0.125 mg/mL EE; Lane 4: DNA + 1 mM $FeSO_4$ + 2% H_2O_2 + 0.25 mg/mL EE; Lane 5: DNA + 1 mM $FeSO_4$ + 2% H_2O_2 + 0.5 mg/mL 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.

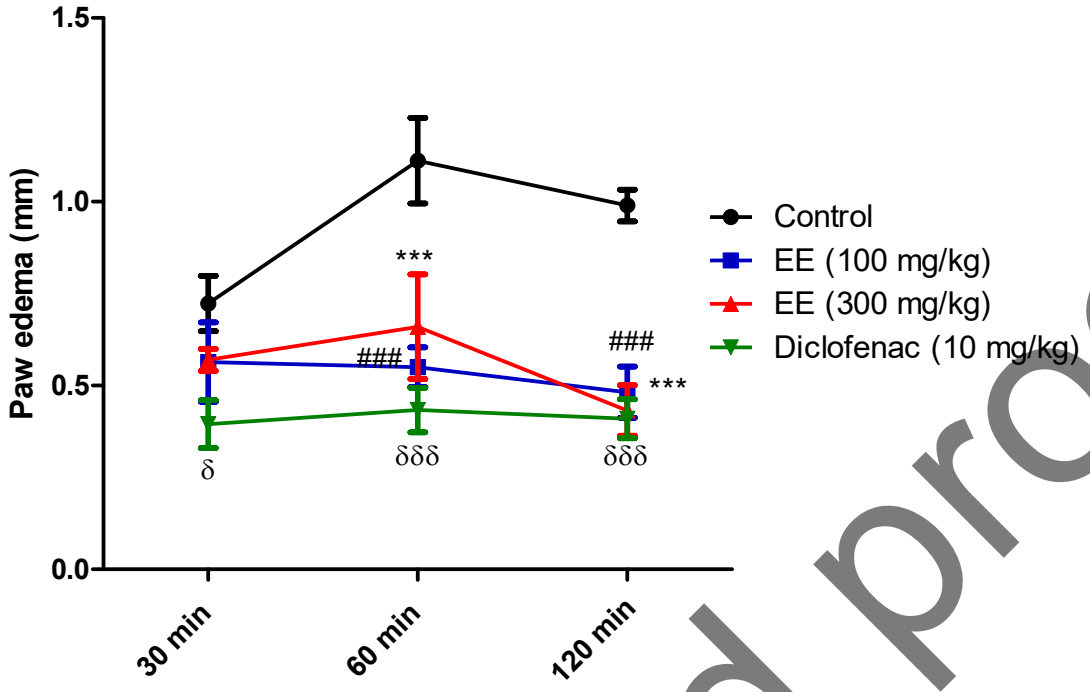

Figure 4. Effect of EE of *V. arctostaphylos* fruits in formalin induced paw edema in mice (n=6). ### p<0.001 EE (100 mg/kg) vs control group, *** p<0.001 EE (300 mg/kg) vs control group, δ p<0.05; δδδ p<0.001 diclofenac (10 mg/kg) vs control group (Two-way ANOVA, post-hoc Bonferroni).