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SOME EDIBLE FOREST FRUITS THEIR IN VITRO ANTIOXIDANT ACTIVITIES, PHENOLIC COMPOUNDS AND SOME ENZYME INHIBITION EFFECTS

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ABSTRACT

The purpose of the study was to analyze the potential health-promoting components of some edible forest species of fruits. These are Service tree (*Sorbus domestica*), Black rosehip (*Rosa pimpinellifolia*), Red rosehip (*Rosa canina*), Bay fruit (*Laurus nobilis*), Cornelian cherry (*Cornus mas*), Autumn-olive (*Elaeagnus umbellate*), and two different blueberry fruits (*Vaccinium arctostaplylos* and *Vaccinium myrtillus*). In the present study, chemical and antioxidant activities, phenolic compounds as well as, inhibition effects on important enzymes (acetylcholinesterase, xanthine oxidase and urease) of fruits were studied. Total polyphenol, flavonoid content, and the in-vitro antioxidant activity were evaluated by DPPH and FRAP. The sugar contents and phenolic compounds of the fruits were analyzed by HPLC-RID and HPLC-UV. Fruits was determined the total polyphenol content (TPC) 1.10 to 19.87 mgGAE/g, total flavonoid content (TFC) 0.01 to 0.80 mgQE/g, DPPH values 0.22 to 6.03 mg/mL and FRAP values 4.24 to 168. 02 µmol $FeSO_4 7H_2O$/ g. All samples were determined various rate fructose and glucose. HPLC-UV revealed fruits contained different phenolic compounds. Inhibition values of the enzymes were expressed as inhibition concentration (IC_{50}: mg/mL or µg/mL). It can be concluded that edible forest fruits are a potential source of antioxidants with therapeutic importance as well a natural enzyme inhibitor.

KEYWORDS:
Antioxidant, acetylcholinesterase, xanthine oxidase, urease, phenolic compounds, HPLC

INTRODUCTION

Non-wood forest products (NWFPs, fruits, wild herbs, mushrooms) are bio-merchandise commonly consumed worldwide. NWFP are typically used in functional and premium class foodstuffs and nutraceuticals [1]. Global interest in the consumption of foods with high levels of functional properties and nutraceutical compounds is gaining momentum [2]. Among these types of foods, fruits are among the most important functional and nutraceutical foods in our diets [3]. The increased consumption of berries that are naturally rich in phenolic compounds is found to be associated with the prevention of cardiovascular diseases, diabetes, cancer, and obesity. Phenolic compounds are widely distributed in such plants where they act as attractants for certain insects, as free radical scavengers, and in defence against ultraviolet radiation, pathogens and predators [2]. In this context, forest fruits are known for being rich in bioactive compounds, including flavonoids, phenolic acids, tannins and anthocyanin's [3]. Today fruit flavours, personal fruit choices, and leading a healthy lifestyle are majör reasons for fruit consumption. Fruits play a significant role in human health as a highly nutritional and functional food. On the other hand, before the development of modern medicine forest fruits were used in the healing of various diseases and are well-known in folk-medicine [4, 5].

For this reason, fruit consumption has been increasing at local and international markets each passing day. Many scientific studies have shown that dietary intake of fruits has several benefits including antioxidant, antibacterial, antiviral, anticancer, immune system regulation, cholesterol lowering, free radical scavenging effects. Fruits also fight against oxidative damage of the cellular macromolecules like DNA, protein and lipid, prevent ulcers, and protect the liver and cardiovascular system [6, 7]. Therefore, the current studies mostly focus on the bioactive components and enzyme activities of fruits and natural products [8].

Around the world, especially in developed counties as well as Turkey, there has been an increasing interest in high antioxidant and anthocyanin rich fruits, which are vitally important for human health, and for the products made from these fruits. Many studies have been carried out on the fruits of plants across the globe; however, a limited number of studies have been carried out in Turkey. The purpose of

our study was to examine the antioxidant capacity, phenolic compositions, anti- acetylcholinesterase, anti- xanthine oxidase, and anti- urease activities of different types of forest fruit plants (service tree, black rosehip, red rosehip, bay fruit, Cornelian cherry, autumn-olive, bilberry and Caucasian whortleberry).

MATERIALS AND METHODS

Chemicals. All the reagents used were of analytical grade. All phenolic acid standards were obtained from Sigma-Aldrich Chemie GmbH (Germany) and Merck (Darmstadt, Germany). Trolox was supplied by AppliChem (Darmstadt, Germany). Folin–Ciocalteu's phenol reagent and TPTZ were purchased from Fluka Chemie GmbH (Switzerland). Jack bean urease, urea, acetohydroxamic acid, sodium nitroprusside, iron(III) chloride hexahydrate (FeCl3.6H2O), 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ), iron(II) sulfate heptahydrate (FeSO4.7H2O), and DPPH (2,2-Diphenyl-1-(2,4,6-trinitrophenyl) hydrazyl) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Sodium acetate, ferric chloride, and glacial acetic acid were obtained from Merck. LC syringe filters (RC-membrane, 0.2 µm) were obtained from Sartorius Minisart RC 15, Sartorius (Darmstadt, Germany).

Fruit material. A total of 8 samples of forest fruits were obtained from the Black Sea region situated in north Turkey, during 2017. The samples were maintained frozen and stored at -18 °C until analysed (Table 1).

Fruit extraction for antioxidant activity, phenolic analysis and enzyme inhibition. The samples were extracted using the method described previously by Can and Baltas [9]. Approximately 5 g of each fruit sample was added to an equal volume (50 mL) of 99% methanol and homogenized in a blender for 3 minutes. The mixture was continuously stirred with a shaker (Heidolph Promax 2020, Schwabach, Germany) at room temperature for 24 h. Particles were removed with filter paper. The final volume of the solution was adjusted with methanol. The methanolic extract was divided into two parts, the first being used for antioxidant tests and enzyme inhibition, the second for phenolic analysis with HPLC.

The second part methanolic extract was evaporated until dryness with a rotary evaporator at 40° C. The residue was dissolved in 15 mL acidified distilled water (pH 2). Liquid–liquid extraction was carried out with 5×3 mL diethyl ether and 5× 3 mL ethyl acetate, consecutively [10]. Both diethyl ether and ethyl acetate phases were pooled and dried by rotary evaporation (IKA-Werke, Staufen, Germany) at 40°C. The pellet was resuspended in 2 mL methanol, filtered with syringe filters (RC-membrane, 0.45 µm), and injected to HPLC.

Fruit extraction for sugars. Fruit extractions were carried out according to the method described by Kafkas et al. [11]. with some modifications. Fruit samples were dried in an oven at 45 °C for one week. Dried samples were powdered by a crusher and approximately 1 g of each sample was weighed. Powdered fruit samples were transferred to a screw cap Eppendorf tube with 20 mL of aqueous ethanol (80% v/v). A reaction mixture was placed in a shaker and shaken at room temperature for 24 h/200 rpm. Particles were removed with filter paper and the liquid part evaporated to dryness with an evaporator. The residue was dissolved with 2 ml of distilled water and filtered before HPLC analysis.

Determination of total phenolic content (TPC) and total flavonoid content (TFC). The TPC was determined using the Folin-Ciocalteau method following Slinkard and Singleton [12]. Briefly, 680 µL of distilled water, 400 µL of 0.5 N Folin-Ciocalteu reagent, 20 µL of various concentrations of gallic acid and samples were mixed and vortexed. After a 3 minute-incubation, 400 µL of Na_2CO_3 (10%) solution was added and vortexed. Then, the mixture was incubated for 2 hours at 20 °C with interrupted shaking. After that, the absorbance of the mixture was measured at 760 nm using a spectrophotometer. Results were expressed as mg gallic acid equivalents (GAE) per g dry weight (mg GAE/g DW).

TABLE 1
Fruit sample name and Latin name

Sample Name	Latin Name	Location
Service tree	*Sorbus domestica* L.	Tokat
Black Rosehip	*Rosa pimpinellifolia*	Gümüşhane
Red Rosehip	*Rosa canina*	Gümüşhane
Cornelian cherry	*Cornus mas*	Samsun
Bay fruit	*Laurus nobilis*	Sinop
Autumn-olive	*Elaeagnus umbellate*	Trabzon
Bilberry	*Vaccinium myrtillus* L.	Trabzon
Caucasian whortleberry	*Vaccinium arctostaphylos* L.	Trabzon

The TFC was determined by Fukumoto and Mazza [13]. Briefly, 0.5 mL samples, 0.1 mL of 10% $Al(NO_3)_3$ and 0.1 mL of 1 M $NH_4.CH_3COO$ were added to a test tube and incubated at room temperature for 40 minutes. Then, the absorbance was measured against a blank at 415 nm. Results were expressed as mg quercetin equivalents (QE) per g dry weight (mg QE/ g DW).

Antioxidant activities; Ferric reducing antioxidant power (FRAP) and DPPH % SC_{50} scavenging activity assay.

The FRAP assay was conducted as described by Benzie and Strain [14]. The fresh FRAP reagent was made by adding 100 mL of 0.3 M acetate buffer pH 3.6, 10 mL of 10 mM TPTZ solution in 40 mM HCl and 10 mLof 20 mM $FeCl_3$ in a ratio of 10:1:1 and 12 mL of distilled water at 37 °C. In brief, the reaction mixture consisting of 100 µL of the sample and 3 mL of freshly prepared FRAP reagent was incubated at 37 °C for 4 min and the absorbance was measured at 593 nm against a control. Results were expressed as µmol $FeSO_4.7H_2O$/g DW).

DPPH% scavenging activity was measured according to the method described by Molyneux [15]. Each extract solution (0.75 mL was added to 0.75 ml of a freshly prepared 0.1 mM DPPH solution dissolved in methanol. The mixture was shaken and left to stand at room temperature for 50 min in the dark. The absorbance was read at 517 nm against a control using a spectrophotometer. The values were shown as SC_{50} mg/mL sample representing the concentration of each sample that resulted in 50% scavenging of DPPH radicals.

Analysis of phenolic compounds by HPLC. Sixteen standards of phenolic compounds (Gallic acid, protocatechuic acid, catechin, chlorogenic acid, vanillic acid, caffeic acid, syringic acid, ferulic acid, *p*-cumaric acid, rutin, benzoic acid, *o*-cumaric acid, quercetin, ellagic acid, *t*-cinnamic acid, curcumin) were analyzed using HPLC (Elite LaChrom Hitachi, Japan). The sample was injected into the HPLC system with a reverse phase C18 column (150 mm x4.6 mm, 5µm; Fortis). The mobile phase was a mixture of solvent A (2% AcOH in water) and solvent B (70:30, acetonitrile/water) which was sonicated before stirring and continuously degassed by the built-in HPLC system. The injection volume was 25 µL and the column was kept at 30 °C. The flow rate was kept constant at 1 mL min^{-1} using gradient programming; starting the flow of mobile phase as B (5%) to three minutes, gradually increasing (up-to 15, 20, 25, 40 and 80% at 8, 10, 18, 25 and 35 minutes respectively) and decreasing to 5 % at 40 minutes and left for 10 minutes to equilibrate in the column. Phenolic profile was determined according to Can and Baltas [9].

In-vitro anti-acetylcholinesterase, anti-xanthine oxidase and anti-urease assay. Sample extracts were subjected to the method by Ellman [16] to evaluate their potency to inhibit the acetyl cholinesterase (AChE, from Electric Eel, (Sigma–Aldrich, St. Louis, USA). As a control, an identical solution of the enzyme without the inhibitor was processed using the same protocol. Donepezil hydrochloride was used as a positive control. All processes were assayed in triplicate.

The anti-xanthine oxidase activity was determined using a slight modification according to the methods by Hayashi [17]. A well known XO inhibitor (XOI), allopurinol (Sigma–Aldrich, St. Louis, USA) was used as a positive control for the inhibition test. The IC_{50} values of extracts were determined as the concentration of extract that gives 50% inhibition of maximal absorbance.

Urease catalyzes the hydrolysis of urea into carbon dioxide and ammonia. The production of the ammonia was measured using the indophenol method by Weatherburn [18]. Acetohydroxamic acid was used as standard inhibitor. To calculate IC_{50} values, different concentrations of each extracts and standards were assayed at the same reaction conditions. The inhibition concentrations of the extracts (IC_{50}) were calculated from the dose response curve, which reduced absorbance by 50%.

Statistical Analysis. Antioxidant, phenolic compounds and enzyme inhibition data were carried out by three replicates (n = 3), and values were presented as the mean of three replicates. The SPSS version 17.0 statistical software package was used for all statistical analysis.

RESULTS AND DISCUSSION

Total phenolic content (TPC) and total flavonoid content (TFC). In this study, fruits' phenolic compounds were determined as TPC and TFC. TPC is at the same time a marker of a fruit's antioxidant capacity and is generally used as an antioxidant test. The comparative data about total phenolic, total flavonoid and other antioxidant activity content in wild berries are presented in Table-2. Flavonoid, phenolic and triterpenes are natural secondary metabolites that exist in fruits and plants [19]. In this study, fruit flavonoid compounds were determined as TFC spectrophotometric assay. The results are shown in Table 2. DPPH and FRAP assays' results are shown in Table 2.

Total polyphenol and flavonoid of some edible fruits were determined. TPC levels varied widely, between 1.90 and 19.868 mg GAE/g. The total phenolic content in Cornelian cherry was found to be higher and service tree was found to be lower than that in other fruits. Black rosehip and red rosehip, also showed high TPC, with values of 18.867 and

5.909 mg GAE/g, respectively. A study by Tural and Koca [20] found the TPC in twenty-four Cornelian cherry species to be between 2.81-5.79 mg GAE/g FW. The TPC of Cornelian cherry fruits from Bosnia-Herzegovina, as studied by Drkenda et al. [21] was found to be 119.10- 230.63 mg 100 g-1 FW. On the other hand Roman Stănilă and Stănilă [22] who studied the total polyphenol content of the eight rosehip fruit species, reported between 326 and 575 mg/100 g, data which are lower than in our study [22]. The fruits of bilberry, Caucasian whortleberry and services tree showed a significantly lower phenolic content than fruits of the rosehips species. In our study, the fruit of autumn olive also showed high level of phenolic content, 1.905 mg GAE/g (Table-2). [23] also studied the TPC of different genotypes of autumn olive. The authors reported that the total phenolic content of autumn olive species varied from 168.9 to 258.1 mg GAE/100 g. Other studies have also reported that the total phenolic contents of autumn olive (*Elaeganus umbletta*) extracted with water, methanol, acetone and n-hexane were found 20.0, 18.6, 18.2, 16.3 mg GAE/g, respectively [24]. The TPC amount of autumn olive in our study is similar with this literature. Finally, the TPC amounts of some fruit species are not similar with the literature. The difference observed in TPC can be attributed to the different locations as well as the fact that synthesis phenolic compounds are affected by various abiotic and biotic factors, including temperature, because of differences between day and night temperatures, irradiation, herbivory, and pathogenic infection [25].

The total flavonoids (TFC) amount of fruits was found between 0.005 and 0.866 mg QE/g in this study. Black rosehip samples exhibited the highest amount of TFC (0.866 mg QE/g) and service tree the lowest was 0.005 mg QE/g. Roman et al. [22] reported that TFC amount of the R. *canina* was 1.63 mg/g. TFC of services tree were found 0.005 mg QE/ g in this study. One study reported that the total flavanoid content of services tree fruits collected from Croatia varied between 6.8 and 37.0 mg QE/ g dw in immature exocarp, immature mesocarp, mature exocarp and mature mesocarp [26]. Other studies have also reported that total flavanoid content of services tree fruit is 18.56 µg QE/ mg [27]. This discrepancy is explainable by different vegetative conditions or by differences in the extraction and sample preparation procedures used in studies [28]. The TFC of autumun-olive 0.552 mg QE/g was found in this study Islaq et al. [24] compared to the TFC of autumun-olive that were obtained by four different extraction methods (water, methanol, aceton and n-hexane). The authors were reported to be 3.8 mg QE/g for water, 3.2 mg QE/g for methanol, 1.5 mg QE/g for acetone, and 3.4 mg QE/g for n-hexane [24]. These results showed that the phenolic and flavanoid content of berries are affected by extraction procedure. This may be attributed to the different flavanoid content in the sample.

TABLE 2
Antioxidant Activity of Fruit Samples

Samples	TP (mgGAE/g sample)	TF (mg QE/g sample)	DPPH SC50 (mg/mL)	FRAP (µmolFeSO4.7H2O/g)
Service tree	1.128±0.04	0.01±0.00	6.03±0.01	4.27±0.03
Black Rosehip	18.87±0.10	0.87±0.01	0.27±0.01	151.37±1.15
Red Rosehip	5.91±0.02	0.81±0.02	1.23±0.01	91.33±0.88
Bay fruit	2.60±0.03	0.14±0.01	5.27±0.03	4.24±0.12
Cornelian cherry	19.87±0.27	0.59±0.01	0.22±0.01	168.02±7.41
Autumn-olive	1.91±0.03	0.55±0.01	4.21±0.01	8.92±0.06
Bilberry	2.06±0.01	0.72±0.01	2.45±0.01	30.00±0.08
Caucasian whorthberry	1.94±0.01	0.80±0.01	3.60±0.02	21.98±0.02
Trolox			0.004±0.00	

TABLE 3
Sugar content of fruit samples

Sample	%Arabinose	%Ribose	%Fructose	%Glucose	%Sucrose	%Maltose
Service tree	N.D.	N.D.	4.82	3.45	N.D.	0.45
Black rosehip	1.10	1,14	2.57	2.23	1.30	N.D.
Red rosehip	0.66	1,11	4.85	3.53	0.67	N.D.
Bay fruit	N.D.	N.D.	1.71	1.03	N.D.	0.48
Cornelian cherry	N.D.	N.D.	2.00	2.50	0.57	N.D.
Autumn-olive	N.D.	N.D.	4.70	2.34	N.D.	N.D.
Bilberry	N.D.	N.D.	6.80	7.10	N.D.	N.D.
Caucasian whortleberry	N.D.	N.D.	5.00	4.96	N.D.	N.D.

*N.D. : not detected, * Galactose, Theralose, Melebiose and Melesitose couldn't be determined.

Antioxidant activity. Molecules known as antioxidants prevent oxidation in living organism by decreasing free radicals or by eliminating these [29]. There are many methods of measuring the antioxidant capacity in natural products [30]. Antioxidant capacity of fruits and plants are affected by different mechanisms of action of their antioxidant constituents. A single method is usually insufficient when determining antioxidant activity. Therefore, this capacity could be evaluated by a variety of methods pertaining to different mechanisms. As a result of DPPH and FRAP assays were used to properly evaluate the antioxidant capacity of dry fruit sample (Table 2). FRAP values 4.27 to 168.02 (µmolFe $O_4.7H_2O$/g DW) and DPPH values ranged from 0.22 to 6.03 mg/mL. The DPPH and FRAP results proved that the Cornelian cherry fruits especially possess a higher antioxidant capacity compared to the other fruits. Findings from Table 2. Illustrate that the lowest antioxidant activity according to FRAP and DPPH methods is seen in service tree. When forest fruits are examined from Table 2, it seen that all forest fruits are an important antioxidant source. Hassanpour et al. [31] investigated the antioxidant activity of the Cornelian cherry (*Cornus mas* L.) fruits naturally growing in East Azerbaijan and Iran. The DPPH antioxidant activity was determined as 82.87% in fresh fruit samples. A study conducted in Iran analyzed the antioxidant capacities of bay fruits using the DPPH method with three different extraction methods (Ultrasonic, Maceration and Soxhlet).

Sugar Content. Organic acids and sugars contribute greatly to the taste and flavour quality of fruits [32]. Among these compounds, fructose is especially significant. Sugar compounds and amounts are shown in Table 3. According to this, fructose and glucose sugar were determined as the major monosaccharide in all fruits. In addition to these sugar compounds, arabinose, ribose, sucrose and maltose were also found in various rates in different fruits. While the highest fructose to glucose ratio among the berries was identified in bilberry, the lowest fructose to glucose ratio was found in bay fruit. Glucose, fructose and sucrose compounds were found in the fully ripened fruits as a result of the sugar analysis of bilberry and Caucasian whortleberry collected in the Black Sea Region. It was reported that the compounds found in Caucasian whortleberry were 25.32% fructose, 26.20% glucose and 1.02 sucrose. These ratios are reported for bilberry as 32.90% fructose and glucose and 1.81% sucrose [33]. We could not detect any sucrose in the fruits of bilberry and Caucasian whortleberry.

Phenolic compounds. The phenolic compounds in fruits are crucial with regards to their functions in promoting human health, their effects on taste and odour formation, and their involvement in colour formation and changes, and also their antimicrobial and anti-oxidative effects. In this study, among the fruits investigated by the RP-HPLC-UV analysis, the 16 phenolic acids were determined qualitatively and quantitatively. The results are presented in Table 4. Phenolic compounds are ubiquitous in both plants and fruits, and when plant and fruit foods are consumed, these phytochemicals contribute to the intake of natural antioxidants in human diets. The phenolic contents of the fruits were determined by HPLC-UV. The results indicated that gallic acid and protocatechuic acid were detected in all fruits except this bilberry and Caucasian whortleberry, whereas no curcumin was detected in any fruit. Other compounds were found at different levels in some berries. Catechin, *t*-cinnamic acid, rutin and elangic acid were found dominant compound in services tree, black rosehip, bay fruit and autumn-olive respectively. While, chlorogenic acid was found to be the dominant compound in bilberry and Caucasian whortleberry, gallic acid was found to be the dominant compound in black rosehip and Cornelian cherry.

Demir et al. [32]. reported that as a result of the qualitative analysis they performed on rosehip berries (*R. canina*, *R. dumanis, R. gallica, R. dumalis subsp. boissieri* and *R. hirtissima*) gallic acid and cathecin are the most dominant compounds [30]. The literature is largely parallel with this study. However, authors found higher amounts of gallic acid and catechin compered to our study. Compared with this study, chlorogenic acid was not found to be a dominant compound, while gallic acid was determined dominant compound for cornelllian berry. However, authors found higher amount of gallic acid and chlorogenic acid compared to our study. According to Hakkinen and Torronen [34] the reason for this is that the hybrid of the same plant variety demonstrated differences in the synthesis of the phenolic compounds [34].

Gallic acid was determined as a major constituent in Cornelian cherry fruits as well. It was also reported that gallic acid decreases the peroxidation in human brain phospholipids [35]. Ellagic acid was determined as a major constituent in autumn-olive fruits though. In vitro studies have revealed that ellagic acid possesses anticancer effects on breast, prostate and pancreatic cancer cells [36]. Catechin belonging to a group of flavonoids was determined as a major constituent in autumn olive fruits. However, catechin was determined as a routine major constituent in bay fruit.

Anti-acetylcholinesterase, anti-xanthine oxidase and anti-urease assay. This study has examined the enzyme inhibition activities (acetylcholine esterase, xanthine oxidase and urease) of methanol fruit extracts. The results are shown in Table 5. Enzymatic activities such as those of acetylcholine esterase, xanthine oxidase and urease exhibited by

TABLE 4
Phenolic compounds of fruit samples

Standard	Service tree	Black rosehip	Red rosehip	Bay fruit	Cornelian cherry	Autumn-olive	Bilberry	Caucasian whortleberry
Gallic acid	0.06±0.00	2.17±0.02	1.41±0.05	0.14±0.00	12.60±0.07	0.19±0.00	N.D.	N.D.
Protocatechuic acid	2.55±0.09	1.46±0.001	8.69±0.17	3.73±0.31	9.88±1.30	1.79±0.03	N.D.	N.D.
Catechin	14.94±0.40	1.17±0.001	3.30±0.72	7.97±0.16	N.D.	N.D.	N.D.	N.D.
Chlorogenic acid	1.50±0.03	N.D.	N.D.	N.D.	0.81±0.00	N.D.	13.96	19.98
Vanillic acid	0.27±0.08	N.D.	1.32±0.05	0.78±0.18	N.D.	1.41±0.04	N.D	N.D
Caffeic acid	1.27±0.06	N.D.	N.D.	N.D.	N.D.	N.D.	8.25	2.76
Syringic acid	N.D.	N.D.	N.D.	3.41±0.05	N.D.	2.06±0.02	2.31	0.67
Ferulic acid	0.17±0.01	N.D.	N.D.	N.D.	0.92±0.19	2.64 ±0.00	0.25	0.87
p-cumaric acid	0.07±0.00	N.D.	N.D.	2.12±0.01	0.07±0.02	N.D.	N.D.	N.D.
Rutin	N.D.	N.D.	N.D.	14.19±0.19	N.D.	N.D.	0.22	0.54
Benzoic acid	0.87±0.01	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
o-cumaric acid	N.D.	N.D.	0.03±0.00	N.D.	N.D.	N.D.	N.D.	N.D.
Quercetin	2.60±0.05	1.58±0.01	N.D.	3.14±0.32	3.34±0.18	N.D.	N.D.	N.D.
Ellagic acid	N.D.	N.D.	N.D.	N.D.	N.D.	28.22±0.03	N.D.	N.D.
t-cinnamic acid	N.D.	N.D.	14.52±0.10	5.06±0.08	N.D.	4.75±0.01	N.D.	N.D.
Curcumin	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.

***N.D**.=Not detected, ***Results=** µg phenolic compounds/g samples

TABLE 5
Anti-acetylcholinesterase, anti-xanthine oxidase and anti-urease acitivity of fruit samples

Samples	Inhibition of acetylcholinesterase IC_{50} (mg/mL)	Inhibition of xanthine oxidase IC_{50} (mg/mL)	Inhibition of urease IC_{50} (µg/mL)
Service tree	5.41±0.02	2.98±0.06	3.82±0.02
Black Rosehip	0.68±0.01	0.29±0.03	0.09±0.01
Red Rosehip	1.13±0.01	0.97±0.01	0.74±0.02
Bay Fruit	2.55±0.03	1.92±0.01	1.59±0.01
Cornelian cherry	0.09±0.01	0.02±0.01	0.03±0.01
Autumn-olive	3.63±0.01	2.71±0.01	3.39±0.03
Bilberry	3.89±0.03	2.77±0.03	2.13±0.03
Caucasian whortleberry	4.11±0.02	3.59±0.03	3.68±0.07
Donepezil (µg/mL)	17.13±0.02		
Allopurinol (µg/mL)		0.52±0.01	
Acetohydroxamic Acid (µg/mL)			25.09±0.02

plant and fruit based actives have been mostly observed from phenolic and flavonoid compounds. The results of enzyme inhibition are shown in Table 5. It was detected that the amount of the anti-AChE activity varied in fruits. Acetylcholine inhibitors are commonly used in the treatment of glaucoma and Azheimer's disease [37]. Our results have revealed that fruits, especially the Cornelian cherry fruit could be utilized as a natural acetylcholine inhibitor.

The second enzyme, xanthine oxidase (XO), catalyzes the conversion of hypoxanthine to xanthine and of xanthine to uric acid in the metabolism of purines degradation. Since the water solubility of uric acid is rather low, it excessively accumulates especially in the kidneys and joints. The high level of uric acid in the blood inhibits the formation with acetylcholine inhibitors in the body. It is reported that commercially available inhibitory drugs have numerous side effects [38]. For this reason, inhibitors with minimum side effects should be provided. In this study, the anti-xanthine oxidase activity was examined in fruits, and among the fruits, the Cornelian cherry (*Cornus mas* L.) fruit was revealed to be a stronger inhibitor compared to the other fruits.

The third enzyme, urease is a vitally crucial nickel- dependent enzyme which catalyzes the hydrolysis of urea to ammonia and carbon dioxide in the luminal bacterial metabolism. The findings have shown that the Cornelian cherry (*Cornus mas* L.) fruits possess a higher anti-urease activity compared to the other fruits. The highest activity was observed in the Cornelian cherry (*Cornus mas* L.) fruits in all three enzymes. Another study was foundXOI and urease effects on Pyrus elaeagnifolia pears and the IC_{50} values to be 10.75 and 0.97 mg/mL respectively [38]. In our study urease effect red rosehip IC_{50} value 0.74 mg/mL was found. In another study it was reported that aqueous extract of *Diospyros lotus* L. (*Plum Persimmon*) lotus exhibited highly efficient anti- AchE activity and anti-urease 312 with an IC_{50} value of 16.75 and 1.55 mg/mL respectively [39]. In our study all the fruits were found to be potent inhibitors for three enzymes.

CONCLUSIONS

Phytochemical profiling of some forest fruits evaluated in this study revealed a diverse range of antioxidant activity, polyphenolic compounds, sugar content and some biological activities. In conclusion, all forest fruits with high antioxidant activities, are quite rich in phenolic composition and were seen to exhibit high enzyme inhibitions. In this respect, there appears to be a linear relationship between antioxidants and enzyme inhibitions. This study has revealed that the daily consumption fruits are not only an important part of a healthy diet and but also help to prevent the development of many diseases. As a results it can be concluded that edible forest fruits are a potential source of antioxidants with therapeutic importance as well as powerful natural enzyme inhibitor source.

ACKNOWLEDGEMENTS

The authors declare no existing conflict of interest.

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Received: **20.12.2018**
Accepted: **21.05.2019**

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