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In vitro Antidiabetic Activities of Two *Sorbus* Species

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ABSTRACT

Fruits of several *Sorbus* species (Rosaceace) are used both in traditional medicine as antidiabetic, antiinflammatory, diuretic, vasoprotective and in foods. In this study, *in vitro* antidiabetic activities of water extracts of *Sorbus aucuparia* L. (rowan tree) and *Sorbus torminalis* L. Crantz (wild sevice tree) fruits were investigated by measuring inhibitory potentials on α-glucosidase and pancreatic α-amylase activities, the most important digestive enzymes. Also, the total phenolic and flavonoid contents of the fruits were determined to evaluate the association between phenolic content and antidiabetic activity. *S. torminalis* and *S. aucuparia* extracts exhibited strong α-glucosidase inhibitory activity, more effective than that of standard drug acarbose. However, *S. torminalis* has shown moderate inhibitory effect against α-amylase while *S. aucuparia* exhibited weak inhibition. The total phenolic and flavonoid contents of the fruits were correlated with andiabetic activities. It has been suggested that antidiabetic effects of the fruits may be due to phenolic compounds present therein. Therefore, *S. aucuparia* and *S. torminalis* fruits might be potential sources of antidiabetic compounds.

Keywords: *Sorbus aucuparia, Sorbus torminalis*, antidiabetic activity, α-amylase, α-glucosidase.

INTRODUCTION

Diabetes mellitus is a group of metabolic diseases (1). Type 2 diabetes mellitus contains β-cell dysfunction and insulin resistance. When the β-cell function decreases over time, fasting blood glucose and postprandial glucose levels begin to rise and remain out of control (2). In the world wide, prevalence of type 2 diabetes is increasing due to lifestyle-related risk factors such as smoking, obesity, poor diabetes and physical inactivity (1). The increased prevalence of type 2 diabetes has led to the development of many new approaches in the treatment of hyperglycemia. The purpose of these treatments is to reduce and maintain glucose concentrations as normal as possible and thus prevent development of complications (3). Sample treatments include α-glucosidase inhibitors (AGIs; acarbose, miglitol and voglibose) to reduce the absorption of carbohydrates in the intestine and control postprandial hyperglycemia. Acarbose inhibits both α-amylase (EC 3.2.1.1) and α-glucosidases (EC 3.2.1.20), thus preventing absorption of starch and other carbohydrates from the intestine also reduces postprandial glycaemia and helps manage diabetes (4). Lately, there has been much interest in the investigations of the natural α-glucosidase inhibitors for diabetes treatment (5).

Nature is a good source of antidiabetic drugs and plants are valuable dietary supplements to improve blood sugar control and prevent long-term complications of type 2 diabetes (2). Polyphenols are naturally occurring compounds found largely in the fruits (especially like grapes, apples, cherries and berries) and vegetables. Several studies revealed that long-time intake of plant polyphenols in diets have a protective effect to development of many diseases such as diabetes (6).

The genus *Sorbus* mostly distributed in Northern Hemisphere, comprises about 250 species of trees and shrubs. Fruits of several *Sorbus* species (berries) included *S. domestica*, *S. aucuparia* and *S. torminalis* from family Rosaceace are consumed as food sources and used as traditional medicine (7). Also, *Sorbus* species are called 'uvez' in Turkish, which have been used as traditional medicinal

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plants for various purposes in Turkish folk medicine (8). *S. domestica* fruits are consumed by the local population in Greece, not only as a nutritious food, but also traditionally as an antidiabetic agent (9). In this study, we investigated *in vitro* inhibitory effects of two *Sorbus* species on α-glucosidase and α-amylase activities. The antidiabetic activities and amount of total phenolics of *S. aucuparia* and *S. torminalis* fruits has been determined, comparatively. Although there are limited number of studies on *S. aucuparia* fruits, there is no study showing the antidiabetic effects of *S. torminalis*.

MATERIAL AND METHODS

Chemicals and Reagents

α-Amylase, α-glucosidase, acarbose, 3,5-dinitrosalicylic acid (DNS), Folin-Ciocalteu reagent, gallic acid, p-nitrophenyl α-D-glucopyranoside (PNPG) and starch purchased from Sigma Chemical Co. (St. Louis, MO, USA). Catechin was purchased from Fluka Chemical Co. (Buchs, Switzerland). All other chemicals or reagents were of analytical grade.

Preparation of Extracts

The fruits of *S. aucuparia* and *S.torminalis* were obtained from Black Sea Region and Istanbul, respectively. Decoctions are one of the most consumed drinkable forms of plants (10). For this reason, the fruit extracts were obtained by using decoction method. After the seeds were removed, fruits were dried in the shade. To prepare the water extracts, 15 g of the fruits were refluxed with distilled water for 3 hours. The extracts were filtered and the solvent was evaporated (Buchi, Switzerland) to dryness under reduced pressure. The fruits extracts were stored in -20°C until needed. For the biochemical assays, the extracts were dissolved in distilled water.

Determination of α-Glucosidase Inhibitory Activity

The α-glucosidase inhibitory effects of the fruit extracts were evaluated using a procedure described by Bothon et al. (11). For the α-glucosidase assay, 25 µL of the fruit extract was mixed with 75 µL of 0.1 M sodium phosphate buffer (pH 6.8) and 50 µL of α-glucosidase solution (1 U/mL) and preincubated at 37°C for 10 minutes. After incubation, 50 µL of substrate solution (5mM PNPG) was added to the reaction mixture and the absorbance change at 405 nm was measured at 37°C for 10 minutes using a microplate reader. Acarbose was used as a standard and replacing the extract with distilled water was used a control. The inhibitory activities of the extracts were identified according to the following formula:

$$\%\,Inhibition = \left(1 - \frac{Reaction\ rate\ of\ sample\ at\ 405\ nm}{Reaction\ rate\ of\ control\ at\ 405\ nm}\right) \times 100$$

Determination of α-Amylase Inhibitory Activity

The inhibition of α-amylase by the *Sorbus* fruits was determined using the DNS method (12). Briefly, 10 µL of each extract were preincubated with 50 µL of α-amylase solution (3 U/mL) and 40 µL of 0.1 M sodium phosphate buffer (pH 6.8) at 25°C for 10 minutes. The reaction was initiated by adding 50 µL starch solution (0.75%). After 5 minutes, the reaction was stopped by adding 75 µL of DNS color reagent (96 mM DNS and 5.31 M potassium sodium tartarate in 2 M NaOH). The mixtures were heated at 85°C for 15 minutes. After cooling, the mixture was diluted 4-fold with distilled water and absorbance was recorded at 540 nm. Acarbose was used as a standard and control was prepared without inhibitor. The inhibitory activities of the extracts were identified according to the following formula:

$$\%\,Inhibition = \left(1 - \frac{Absorbance\ of\ sample\ at\ 540\ nm}{Absorbance\ of\ control\ at\ 540\ nm}\right) \times 100$$

Determination of Total Phenolic and Flavonoid Compounds

Total phenolic and flavonoid contents of the extracts were determined using the Folin-Ciocalteu (13) and the aluminum chloride (14) methods, respectively. For the determination of total phenolics, 5 µL of fruit extract was mixed with 225 µL of distilled water, 5 µL of 2 N Folin-Ciocalteu reagent (previously diluted with distilled water 1:2; v:v) and 15 µL of 2% Na_2CO_3 solution. The mixture was incubated in dark for 2 hours at room temperature. After incubation, absorbance was measured at 760 nm. Total phenolic contents were determined using equation of standard regression curve which obtained by gallic acid solution and were expressed in mg of gallic acid equivalents (GAE).g extract^{-1}.

For the determination total flavonoids, 25 µL of fruit extract was mixed 125 µL of distilled water and 7.5 µL of 5% $NaNO_2$ solution then incubated for 6 minutes. Then, 15 µL of 10% $AlCl_3$ solution was added. After 5 minutes incubation at room temperature, 50 µL of 1 M NaOH solution and 27.5 µL of distilled water was added. The absorbance was recorded at 510 nm. Total flavonoid contents were determined using equation of standard regression curve which obtained by catechin solution and were expressed in mg of catechin equivalents (CE).g extract^{-1}.

Statistical Analysis

The results were evaluated using unpaired t-test with NCSS statistical computer package (NCSS, Kaysville, UT, USA) and the differences were considered significant at $p<0.05$.

RESULTS

In this study, the inhibitory effects of two *Sorbus* species and acarbose on α-glucosidase and α-amylase activities were investigated. It was found that *S. torminalis* and *S. aucuparia* showed strong and dose dependent inhibitory activities against α-glucosidase (Figure 1). The half-maximal inhibitory concentration (IC_{50}) values of the *Sorbus* extracts and acarbose are presented in Table 1. Comparison of the IC_{50} values revealed that the inhibitory effects of both *S. torminalis* and *S. aucuparia* extracts on α-glucosidase were approximately four and two fold higher than that of acarbose, respectively. As shown in Figure 2, *S. torminalis* exhibited 75.32±2.80% α-amylase inhibitory activity at 0.8 mg.mL^{-1} concentration while *S. aucuparia* exhibited only 22.08±1.17 % inhibition at same concentration.

Also, the total phenolic and flavonoid contents of the extracts are shown in Table 2. The results showed that *S. torminalis* water extract had the highest total phenolic and total flavonoid contents. These results demonstrate that there was a high correlation between the antidiabetic activity and the phenolic contents.

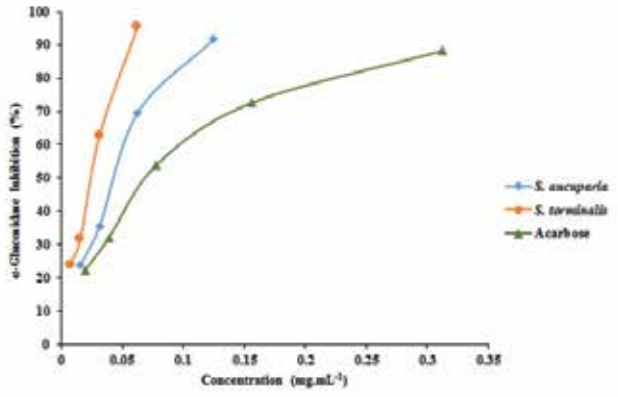

Figure 1. Effects of *Sorbus* extracts and acarbose on α-glucosidase activity

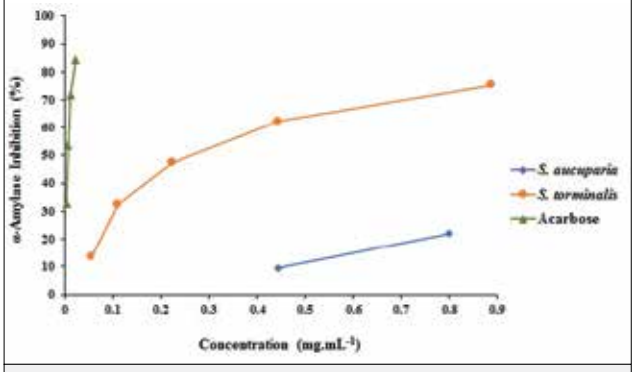

Figure 2. Effects of *Sorbus* extracts and acarbose on α-amylase activity

DISCUSSION

One of the therapeutic approaches in the treatment of diabetes mellitus is reduction of postprandial hyperglycemia (15). The rate of starch digestion is the most important factor affecting of blood glucose level. Since α-glucosidase and α-amylase have a crucial function in carbohydrate hydrolysis, inhibition of these enzymes is one of the most therapeutic strategy for the treatment of diabetes (5). In this study, we evaluated α-glucosidase and α-amylase inhibitory activities of two *Sorbus* species. Our results showed that *S. torminalis* and *S. aucuparia* strongly inhibited α-glucosidase activity indicates tested species have antidiabetic effects. However, *S. torminalis* and *S. aucuparia* showed moderate and weak inhibitory effect on α-amylase, respectively. In literature, there have been limited studies on the antidiabetic effects of *S. aucuparia* fruits while no studies showing antidiabetic activity of the *S. torminalis* fruit were found. In these studies, the antidiabetic effect of *S. aucuparia* fruit extract was reported by measuring α-amylase inhibitory activity (16) and α-glucosidase inhibitory activity (1). These results are consistent with the data obtained from this study. Also, antidiabetic potentials of different *Sorbus* species (S. *decora* and *S. tianschanica*) have been reported in diabetic animal models (17,18).

Table 1. α-Glucosidase and α-amylase inhibitory activities of the extracts and acarbose

	Inhibitory activity (IC_{50} mg.mL^{-1})	
	α-amylase	**α-glucosidase**
S. aucuparia	ND	0.050±0.0005[a]
S. torminalis	0.307±0.0158[a]	0.027±0.0006[b]
Acarbose	0.006±0.0002[b]	0.086±0.0027[c]

Data are presented as the mean of three replicates ± standard deviation. Different superscript letters in the same column indicate a significant difference ($p<0.05$). IC50; The inhibitory concentration of the extract or acarbose required to inhibit the activity of the enzyme by 50%. IC50 values were calculated from dose-response curves using Microsoft Excel. All concentrations are the final extract concentrations in the reaction mixture. ND; Not determined.

Table 2. Total phenolics contents (TPC) and total flavonoid contents (TFC) of the extracts

	TPC (GAE.g extract^{-1})	TFC (CE.g extract^{-1})
S. aucuparia	19.13±0.76	9.62±0.27
S. torminalis	24.21 0.61	15.69±0.55

Data are presented as the mean of three replicates±standard deviation.
GAE.g extract^{-1}; mg gallic acid equivalents.g extract^{-1}.
CE.g extract^{-1}; mg catechin equivalents.g extract^{-1}.

In this study, we also determined the total phenolic and flavonoid contents of the fruit extracts. It was found that there was a high correlation between phenolic contents and *in vitro* antidiabetic acitivity. Antidiabetic effects of polyphenolic compounds have been shown in numerous studies (6,19). It has been suggested that hypoglycemic effects of fruits and vegetables may stem from the insulin-like or insulin releasing activities of phenolic compounds present therein (2). Also, α-glucosidase and α-amylase inhibitory potentials of various plant polyphenols such as catechins, diacetylated anthocyanins and alkaloids have been reported in several studies (2,6,20). Phenolic composition of *S. torminalis* and *S. aucuparia* fruits have been shown in previous studies (21,22). Based on the correlation between the results of the assays, we can say that the phenolic compounds in the fruit extracts are responsible for its antidiabetic activity.

In recent work, we demonstrated that the extracts from *Sorbus* fruits especially *S. torminalis*, potently inhibit α-glucosidase and α-amylase *in vitro*. It is reasonable to hypothesize that consumption of *Sorbus* fruits may reduce intestinal absorption of sugars via inhibition of these digestive enzymes. Also, these fruits can be a potential source of natural antidiabetic agents. These findings may scientifically explain some uses of this species in folk medicine as an antidiabetic agent.

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