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Original Article

In vitro investigation of *Sorbus domestica* as an enzyme inhibitor

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ABSTRACT

Background and Aims: Finding new therapeutic enzyme inhibitors by investigating especially medicinal plants is an important research area. The fruits and leaves of *Sorbus domestica* (service tree) are used as food and folk remedies due to astringent, antidiabetic, diuretic, antiinflammatory, antiatherogenic, antidiarrhoeal, vasoprotective, and vasorelaxant activities, and also used commercially as a vitamin and antioxidant. In this study, the therapeutic effect of *S. domestica* against diabetes, Alzheimer's disease, aging, and hyperuricemia was investigated.
Methods: α-Glucosidase, α-amylase, acetylcholinesterase (AChE), butyrylcholinesterase (BChE), elastase and xanthine oxidase (XO) inhibitory activities of the fruit extract from *S. domestica* were measured.
Results: The extract showed inhibitory activity against α-glucosidase, α-amylase, BChE, elastase, and XO whereas AChE inhibitory activity of the extract could not be determined. Moreover, the inhibition effects of the extract against α-glucosidase and elastase were more effective than the standard drugs acarbose and ursolic acid, respectively.
Conclusion: *S. domestica* can be evaluated as a potential source for a new therapeutic agent.

Keywords: α-glucosidase, α-amylase, acetylcholinesterase, butyrylcholinesterase, elastase, xanthine oxidase

INTRODUCTION

The discovery of enzyme inhibitors is an active area of research in biochemistry and pharmacology. There are many drugs that act as reversible/irreversible enzyme inhibitors (Balbaa & Ashry, 2012). α-Amylase (EC 3.2.1.1) and α-glucosidase (EC 3.2.1.20) are carbohydrate-processing enzymes present in the gastrointestinal tract. Postprandial hyperglycemia could be decreased through the inhibition of these enzymes. Therapeutic inhibitors of α-glucosidase such as acarbose have been used for the management of hyperglycemia in diabetes patients (Oboh, Ogunsuyi, Ogunbadejo, & Adefegha, 2016). Acetylcholinesterase (AChE; EC 3.1.1.7) and butyrylcholinesterase (BChE; EC 3.1.1.8) are enzymes that catalyze the hydrolysis of acetylcholine into choline and acetic acid, an essential process for the regeneration of the cholinergic neurotransmission. Cholinesterase inhibitors increase the synaptic level of the neurotransmitter and therefore are used in the symptomatic treatment of Alzheimer's disease, which is the most common reason for dementia (Gülçin et al., 2016; Türkan, Huyut, Taslimi, & Gülçin, 2019). Elastase (EC 3.4.21.36) is a member of the proteases, which is responsible for the breakdown of elastin as well as collagen, fibronectin, and other extracellular matrix proteins providing elasticity to connective tissues (Azmi, Hashim, Hashim, Halimoon, & Majid, 2014; Shukla, Park, Park, Lee, & Kim, 2017). The inhibitors of elastase have the potential to be cosmetic ingredients in combating skin aging due to their usefulness in preventing the loss of skin elasticity and sagging (Azmi et al., 2014). Xanthine oxidase (XO; EC 1.2.3.2) catalyzes the oxidation

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of hypoxanthine, and this reaction is terminated by the formation of uric acid. The overproduction or underexcretion of uric acid leads to hyperuricemia ending as gout. The use of XO inhibitors is one of the medications to cure gout by blocking the formation of uric acid (Harrad & Amine, 2016).

Plants are perfect sources to find new inhibitor compounds for the treatment of diseases (Balbaa & Ashry, 2012). *Sorbus* genus (Rosaceae) comprises more than 250 species and spread throughout the various parts of the World (Olszewska & Michel, 2009). *S. domestica* is a tree that opens white flowers in May-June and usually 5-10 m in length (Baytop, 1999). The fruit of *S. domestica* is a pome 2-3.5 cm long and has greenish-yellow color (Brus, Ballian, Bogunić, Bobinac, & IdžOjtić, 2011). The leaves and fruits of *S. domestica* are used in traditional medicine for the treatment of various diseases due to their astringent, antidiarrheal, diuretic, anti-inflammatory, anti-atherogenic, vasoprotective, vasorelaxant, and antidiabetic effects, as well as used in food industry (as antioxidant agents in beverages production and sweet/jam production) (Baytop, 1999; Ölschläger, Milde, Schempp, & Treutter, 2004; Termentzi, Kefalas, & Kokkalou, 2006; Kültür, 2007; Olszewska & Michel, 2009). In Turkey, the edible fruits are called "üvez" and "börtlücen" (Kültür, 2007). Previous studies have demonstrated the inhibitory potential of some *Sorbus* species (*S. torminalis* and *S. aucuparia*) on different enzymes (McDougall, Kulkarni, & Stewart, 2009; Boath, Stewart, & McDougall, 2012; Ivanov, Garbuz, Malfanov, & Ptitsyn, 2013; Hasbal, Yilmaz-Ozden, & Can, 2015; Hasbal, Yilmaz-Ozden, & Can, 2017; Olszewska et al., 2019). Also, limited studies have shown that *S. domestica* inhibits the aldose reductase, lipoxygenase, and hyaluronidase (Termentzi, Kefalas, & Kokkalou, 2008; Matczak et al., 2018). In this study, the inhibitory activity of the water extract from *S. domestica* fruits on α-glucosidase, α-amylase, AChE, BChE, elastase, and XO was examined for the first time.

MATERIAL AND METHODS

Chemicals

3,5-dinitrosalicylic acid (DNS), 5,5'-dithiobis(2-nitrobenzoicacid) (DTNB), α-amylase, α-glucosidase, acarbose, acetylthiocholine iodide (ATChI), AChE, BChE, butyrylthiocholine iodide (BTChI), elastase from porcine pancreas, galantamine hydrobromide, *p*-nitrophenyl α-D-glucopyranoside (*p*NPG), *N*-succinyl-Ala-Ala-Ala-p-nitroanilide (STANA), starch, xanthine, and xanthine oxidase (XO) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals were analytical grade.

Preparation of the plant extract

Fruits of *S. domestica* were collected from Bartın in the Black Sea region of Turkey (ISTE 95595). The fruits were air-dried and cut into small pieces. The extraction was performed by the decoction method. Fifteen g of the fruits were refluxed with distilled water for 3 hours. The extract was filtered and the solvent was evaporated (Buchi, Switzerland) to dryness under reduced pressure, then stored in -20°C until needed. For the biochemical assays, the extract was dissolved in distilled water.

α-Glucosidase inhibitory activity

The α-glucosidase inhibitory effect of the extract was investigated according to the procedure of Bothon et al., (2013). Twenty-five µL of the fruit extract were mixed with 75 µL of sodium phosphate buffer (0.1 M; pH 6.8). Then, 50 µL of α-glucosidase solution (1 U/mL) were added, and the mixture was preincubated at 37°C for 10 minutes. Thereafter, 50 µL of *p*NPG solution (5 mM) were added, and the absorbance change was measured at 405 nm. Acarbose was used as a standard, and the control was prepared without inhibitor. The percent inhibition of the enzyme was calculated according to the following formula:

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

α-Amylase inhibitory activity

The inhibition of α-amylase was assayed using the DNS method (Ali, Houghton, & Soumyanath, 2006). Ten µL of the fruit extract were incubated with 50 µL of α-amylase solution (3 U/mL) and 40 µL of sodium phosphate buffer (0.1 M; pH 6.8) at 25°C for 10 minutes before adding the substrate solution. The reaction was initiated by adding 50 µL of 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 measured at 540 nm. Acarbose was used as a standard and the control was prepared without an inhibitor. The percentage inhibition of the enzyme was calculated by the following equation:

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

Cholinesterase inhibitory activity

The inhibitory activities of the extract on AChE and BChE were determined using the method of Ellman, Courtney, Andres, & Featherstone, (1961) with slight modifications. Twenty µL of the extract and 220 µL of Ellman solution (318 mM DTNB, and 955 mM ATChI/BTChI in phosphate buffer; pH 7.5) were mixed, then 10 µL of AChE/BChE solution (0.5 U/mL) were added, and the absorbance change was monitored at 412 nm. Galantamine was used as a standard and the control was prepared without an inhibitor. The percent inhibition of the AChE/BChE was calculated by the following formula:

$$Inhibition\ level\ (\%) = \left(1 - \frac{Reaction\ rate\ of\ sample\ at\ 412\ nm}{Reaction\ rate\ of\ control\ at\ 412\ nm}\right) \times 100$$

Elastase inhibitory activity

The elastase inhibitory activity of the extract was determined according to the method of Moon, Yim, Song, Lee, & Hyun, (2010) with slight modifications. Fifty µL of the extract were preincubated with 50 µL of elastase solution (0.16 U/mL) and 900 µL of Tris-HCl buffer (0.2 M; pH 7.8) at 37°C for 15 minutes before adding the substrate solution. Then, 50 µL of STANA solution (5 mM) were added, and the mixture was incubated at 37°C for 30 minutes. The release of *p*-nitroaniline was measured at 410 nm. Ursolic acid was used as a standard and the control was prepared without an inhibitor. The percentage inhibition of elastase was calculated by the following formula:

$$Inhibition\ level\ (\%) = \left(1 - \frac{Absorbance\ of\ sample\ at\ 410\ nm}{Absorbance\ of\ control\ at\ 410\ nm}\right) \times 100$$

XO inhibitory activity

XO inhibitory activity of the extract was assayed by the method of Kalckar & Shafran (1947) with slight modifications. One mL of the extract was preincubated with 0.1 mL of XO solution (0.04 U/mL) and 2.9 mL of sodium phosphate buffer (50 mM; pH 7.5) at 25°C for 15 minutes before adding the substrate solution. Then, 2 mL of xanthine solution (150 mM) were added, and the mixture was incubated at 25°C for 30 minutes. The reaction was stopped by adding 1 mL of HCl solution (1 N), and the absorbance was measured at 290 nm. Allopurinol was used as a standard and the control was prepared without an inhibitor. The percentage inhibition of the enzyme calculated with the equation:

$$Inhibition\ level\ (\%) = \left(1 - \frac{Absorbance\ of\ sample\ at\ 290\ nm}{Absorbance\ of\ control\ at\ 290\ nm}\right) \times 100$$

Statistical analysis

All samples were analyzed in triplicate. Results are given as means ± standard deviation (SD). The results were evaluated using unpaired t-test with NCSS statistical computer package and differences were considered significant at $p<0.05$. The half-maximal inhibitory concentration (IC_{50}) values were calculated from dose-response curves using Microsoft Excel.

RESULTS AND DISCUSSION

Medicinal plants used in folk medicine are currently being investigated for pharmaceutical, food and nutraceutical preparations (Fotakis et al., 2016). In the present study, we have investigated the inhibitory activity of *S. domestica* extract on α-glucosidase, α-amylase, AChE, BChE, elastase and XO activities in order to find a new compound as an enzyme inhibitor.

Previous research suggests that fruit and vegetable-rich diets are associated with a reduced incidence of type 2 diabetes. Also, the leaves and fruits of *S. domestica* are traditionally used against diabetes in many countries as well as in Turkey (Baytop, 1999; Termentzi et al., 2006; Kültür, 2007). The results showed that the extract of *S. domestica* exhibited a strong α-glucosidase and weak α-amylase inhibitory property compared with acarbose (Table 1). The results obtained from this study coincide with the traditional use of *S. domestica* in diabetes. α-Glucosidase and α-amylase inhibitory activities of some *Sorbus* species have also been demonstrated in limited studies (Boath et al., 2012; Hasbal et al., 2017; Broholm, Gramsbergen, Nyberg, Jäger, & Staerk, 2019).

Enzyme inhibitory approaches are recognized as one of the most efficient strategies for Alzheimer's disease (Balkan et al., 2018). The Food and Drug Administration approved cholinesterase inhibitors, tacrine, rivastigmine, galantamine, and donepezil as the main drugs for the therapy of early and moderate stages of Alzheimer's disease (Vafadarnejad et al., 2018). *S. domestica* water extract showed a weak ability to inhibit BChE whereas the extract did not show AChE inhibitory activity (Table 1). However, the anti-AChE activity of *S. aucuparia* and *S. torminalis* have been reported previously (Hasbal et al., 2015; Mrkonjć et al., 2017; Ozsoy, Yilmaz-Ozden, Serbetci, Kultur, & Akalin, 2017).

Degradative enzymes such as elastase, responsible for the structural changes in the skin are the target of the novel strategies to delay the symptoms of aging (Boran, 2018). One of the most important functions of elastase in combination with matrix metalloproteinases is to provide tissue repair under normal conditions after the wounding process (Azmi et al., 2014). The inhibitory activity of *S. domestica* water extract on elastase is shown in Table 1. The results revealed that the water extract possesses a remarkable inhibitory activity on elastase compared with ursolic acid. In the literature survey, no previous report has been found about elastase inhibitory activities of the *Sorbus* species.

XO inhibitors are used for the treatment of conditions associated with hyperuricemia such as gout (Wang, Zhang, Pan, & Gong, 2015). In this study, the water extract of *S. domestica* exhibited inhibitory activity against XO similar to allopurinol. On the other hand, Olszewska et al., (2019) reported that flower extracts of *S. aucuparia* showed no inhibitory effects towards XO.

CONCLUSION

In this study, α-glucosidase, α-amylase, AChE, BChE, elastase and XO inhibitory activities of *S. domestica* fruits were evaluated for the first time. Our results showed that the water extract from *S. domestica* showed strong inhibitory activity against α-glucosidase and elastase, suggesting that *S. domestica* may be a potential source of natural compounds for the treatment of type 2 diabetes and skin disorders.

Table 1. α-Glucosidase, α-amylase, AChE, BChE, elastase and XO inhibitory activities of *S. domestica* water extract and respective standards

	Inhibitory activity (IC_{50} mg/mL)					
	α-Glucosidase	**α-Amylase**	**AChE**	**BChE**	**Elastase**	**XO**
S. domestica	0.417±0.024*	8.768±0.247*	ND	26.907±1.605*	0.100±0.001*	0.007±0.001*
Acarbose	0.548±0.021	0.120±0.023				
Galantamine			0.008±0.001	0.094±0.003		
Ursolic acid					0.131±0.009	
Allopurinol						0.001±0.001

Data are presented as the mean of three replicates ± standard deviation. *Significant difference $p<0.05$ versus the respective control substances. IC_{50}; The inhibitory concentration of the extract or standards required to inhibit the activity of the enzyme by 50%. IC_{50} values were calculated from dose-response curves using Microsoft Excel. AChE; Acetylcholinesterase, BChE; butyrylcholinesterase, XO; xanthine oxidase.

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Conflict of Interest: The authors have no conflict of interest to declare.

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