ejpmr, 2022,9(9), 06-12

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

INHIBITORY EFFECTS OF ALPHA (A)-AMYLASE AND A-GLUCOSIDASE BY ROOTS AND BARKS OF ULMUS DAVIDIANA VAR. JAPONICA

Man Kyu Huh*

Food Science & Technology Major, Dong-eui University, Busan 47340, Republic of Korea.

***Corresponding Author: Man Kyu Huh**

Food Science & Technology Major, Dong-eui University, Busan 47340, Republic of Korea.

Article Received on 27/06/2022 **Article Revised on 17/07/2022** **Article Accepted on 7/08/2022**

ABSTRACT

Ulmus davidiana var. *japonica* was used for medicinal purposes in oriental medicine. This study was to evaluate and compare the α-amylase and α-glucosidase inhibitory activity of extracts from *U. davidiana* var. *japonica*. α-glucosidase inhibition of water extract for roots ranged from 24.9% at 0.25 mg/ml to 43.7% at 1.0 mg/ml. α-glucosidase inhibition of water extract for barks was ranged from 31.4% at 0.25 mg/ml to 51.6% at 1.0 mg/ml. Although the α-amylase inhibitory activity of the bark was higher than root, there was not significant difference between two groups (p>0.05). The IC_{50} for water extracts of root and bark were 22.89 and 17.96 ug/ml, respectively. α-glucosidase inhibition of water extract for roots ranged from 24.9% at 0.25 mg/ml to 43.7% at 1.0 mg/ml. α-glucosidase inhibition of water extract for barks was ranged from 31.4% at 0.25 mg/ml to 51.6% at 1.0 mg/ml. The mode of inhibition of *U. davidiana* var. *japonica* extracts against both α-amylase and α-glucosidase was confirmed by Lineweaver–Burk plots. Crude water and ethanol extracts of *U. davidiana* var. *japonica* mixed non-competitively inhibited α-amylase and α-glucosidase.

KEYWORDS: α-amylase, α-glucosidase, Inhibitory activity, Lineweaver–Burk plots, *Ulmus davidiana* var. *japonica.*

INTRODUCTION

Ulmus davidiana var. *japonica*, the Japanese elm, is one of the larger and more graceful Asiatic elms, endemic to much of continental northeast Asia, where it grows in swamp forest on young alluvial soils, although much of this habitat has now been lost to intensive forest breeding.[1] *U. davidiana* var. *japonica* resemble *Zelkova serrata*, and the bark is called yupi (Korean), and the root bark is called yugeunpi (Korean). In folk therapy, it has been used as a swelling drug mainly using water with muscle fatigue, and it is widely known in folk therapy because it can be used for various diseases caused by inflammation, spleen, and various diseases of the stomach.[2,3] Especially, its stem and root barks have been used as a traditional medicine for the treatment of edema, mastitis, cancer, inflammation, and rheumatoid arthritis for a long time.[4] However, Kim et al.[5] suggested that ingestion of complementary medicine (elm bark root extracts) can be a cause of acute toxic hepatitis and acute kidney injury. Kim et al.[6] reported that *U. davidiana* has a strong antioxidative effect on lipid peroxidation as well as an inhibitory effect on endogenous NO-induced apoptotic cell death. The antioxidant activity of ethanol or methanol extracts of this species was investigated against several in vitro methods.[7, 8]

Alpha (α)-amylases (EC 3.2.1.1) are a widespread group of enzymes that catalyze the hydrolysis of internal α-1,4-glucosidic linkages and are thus ideally suited to cutting a starch polymer into smaller fragments.[9]

In addition to their potential in biotechnological applications, α-amylases have received increased interest in the past decades in the context of human health.[10] In mammals, digestion of starch by salivary and pancreatic α-amylases leads to linear and branched malto-oligosaccharides.

Alpha (α)-glucosidase (EC 3.2.1.20) hydrolyzes terminal non-reducing (1→4)-linked alpha-glucose residues to release a single alpha-glucose molecule. α-glucosidase is a carbohydrate-hydrolase that releases alpha-glucose as opposed to beta-glucose.[11] These are largely hydrolyzed to glucose by α-glucosidase located in the intestinal mucosa, which then enters the bloodstream by means of facilitated diffusion. Control of postprandial glucose levels, which has proven to be a key factor for the treatment of diabetes and obesity, might be achieved by activity modulation of starch-processing enzymes.[12]

The objective of the present study was to provide an in-vitro study for the potential inhibitory activity of *Ulmus davidiana* var. *japonica* on α-amylase and α-glucosidase enzymes. This inhibition lowers the rate of glucose

absorption through delayed carbohydrate digestion and extended digestion time. I wondered to what extent *U. davidiana* var. *japonica* inhibited the digestive enzyme of glucose. It evaluated in inhibition of α-glucosidase and porcine pancreatic α-amylase activities by its crude aqueous and ethanol extracts with *U. davidiana* var. *japonica*.

MATERIALS AND METHODS

Sample extract

The dry and powder *U. davidiana* var. *japonica* was purchased from an oriental herb market in Seoul, Korea. Samples were divided into two groups: root (500 g) and bark (500 g). Each sample were treated with distilled water or 80% ethanol. The mixture was further stirred with a magnetic bar at 60℃ for 12 hours. The sample was treated with ultrasound at room temperature for 60 minutes. The ultrasound extraction was carried out using an ultrasonic bath (5510, Branson, USA). The mixture was shaken vigorously for one hour at room temperature. Extracted sample was filtered through Whatman No. 4 filter paper. The extract was evaporated to remove solvent under reduced pressure, using rotary vacuum evaporator (N-1001S-W, Eyela, Tokyo, Japan). To get dry powder, samples placed in a low temperature vacuum chamber.

α-amylase inhibitory assay

α-amylase activity was assayed spectrophotometically. The determination of α-amylase inhibitory activity was carried out by quantifying the reducing sugar (maltose equivalent) liberated under assay conditions by the method described Apostolidis and Lee[13] with some modification. The assay mixture containing 25 µl of 50 mM phosphate buffer pH 6.8, 2.5 µl extract and pre-incubated porcine α-amylase (0.25 U/ml) were incubated at 25℃ for 10 min. After pre incubation, 25 µl of 0.5% starch solution was added.The reaction mixtures were then incubated at 25℃ for 10 min. The reaction was terminated with the addition of 150 µl of 90 mM 3,5-dinitrosalicylic acid (DNS) reagent and placed in boiling water bath for 10 minutes. The extract was then cooled to room temperature until use. Absorbance (A) was measured at 540 nm. Acarbose (4,6-Dideoxy-4-([1S]-[1,4,6/5]-4,5,6-trihydroxy-3-hydroxymethyl-2-yclohexenylamino)-maltotriose) (Sigma Aldrich Chemical Co, USA) was used as reference standard (positive control). Control incubations represent 100% enzyme activity and were conducted in a similar way by replacing extracts with vehicle. For blank incubation (to allow for absorbance produced by the extract), enzyme solution was replaced by buffer solution and absorbance recorded. Separate incubation carried out for reaction t = 0 was performed by adding samples to DNS solution immediately after addition of the enzyme. Experiments were performed in triplicate.

The concentration of the extract that inhibits 50% of the enzyme activity (IC_{50}) was calculated. Extracts with high inhibitory activity were analyzed using a series of suitable extract concentrations. IC_{50} values were determined by plotting percent inhibition (Y axis) versus log10 extract concentration (X axis) and calculated by logarithmic regression analysis from the mean inhibitory values. Regression analysis by a dose response curve was plotted to determine the IC_{50} values.

α-glucosidase inhibitory assay

The bioassay method of multiwell plate system was applied for α-glucosidase inhibitory activity assay as described by Deutschlander et al.[14] with some modification. Extracts and catechins were prepared as described above. Briefly, the test compound and 2 mU of Yeast α-glucosidase (Cat. No: G 5003, Sigma Aldrich Chemical Co, USA) was dissolved at a concentration of 0.1 U/ml in 100 mM sodium acetate buffer (pH 5.6). Enzyme source was prepared bovine serum albumin 2000 mg/ml and sodium azide 200 mg/ml in 100 mM sodiumacetate buffer (pH 5.6). The substrate solution, 2 mmol Para-nitrophenyl-α-D-glucopyranoside (pNPG) (Cat. No: N 1377, Sigma Aldrich Chemical Co, USA) was prepared in the same buffer. A total of 20 ul from each extract were diluted to 97 µL in 0.1 M sodium acetate buffer (pH 5.6) and pre-incubated in 96-wellplates at 37℃ for 10 min. The reaction was initiated by adding 3 µL of 3 mM pNPG assubstrate. The plate was incubated for an additional 10 min at 60℃, followed by addition of 100 µL 1 M NaOH to stop the reaction. All test compounds were prepared in DMSO as described above. The final concentrations of extracts and catechins were between 0.03-10 µg/mL and 5–1000 µM, respectively. The final concentration of α-glucosidase was 20 mU/mL. The optical density (OD) of the solution was read using the Microplate Reader (VersaMax, Califonia, USA) at the wavelength 410 nm.

The reaction system without extracts was used as control and system without α-glucosidase was used as blank for correcting the background absorbance. Acarbose was used as reference standard (positive control). Acarbose, known as BAY g 5421, is an α-glucosidase inhibitor that prevents absorption of sucrose and maltose. Extracts of samples at different proportions (100:0, 75:25, 50:50, and 25:75 mg/ml) were performed in triplicate. IC_{50} values were determined by plotting percent inhibition as α-amylase.

Inhibitory analysis

Data was conducted using Microsoft Excel and SPSS 21.0 for Windows (Chicago, IL, USA). A one-way and a two-way analysis of variance (ANOVA) followed by the Tukey post hoc test were used to analyze statistical significance ($p < 0.05$). All analysis was carried out at least in triplicate. The results were expressed as the mean±SD. Significance and confidence level were estimated at $p < 0.05$.

The percent inhibition was calculated as the decolourization percentage of the test sample using the following formula:

Inhibition (%) = (IA-As)/IA×100
Where IA is the absorbance of the 100% initial and As is the absorbance of the sample. IA and As were the values which were subtracted the average absorbance of the blank wells.

Kinetics of inhibition against α-amylase and α-glucosidase

Inhibition modes of the plant extracts against α-amylase and α-glucosidase were determined according to the method described by Kim et al.[15] Briefly, fixed amounts of both α-amylase and α-glucosidase were incubated with increasing concentrations of their substrates (starch and PNPG, respectively) at 37°C for 20 min, in the absence or presence of water extracts (5 mg/ml). Reactions were terminated and absorption measurements carried out as a for ementioned. Amounts of products liberated (reducing sugars as maltose and p-nitrophenol, respectively) were determined from corresponding standard curves and converted to reaction rates according to the following formula.

$$\text{Reaction rate (v) (mg.ml}^{-1}\text{.s}^{-1}\text{)} = \frac{\text{Amount of product liberated (mg.ml}^{-1}\text{)/}}{1200\ (s)}$$

Inhibition types were then determined by Lineweaver–Burk plot (1/v versus 1/[S]) where [S] analysis of data is according to Michaelis–Menten kinetics.[16]

RESULTS

α-amylase inhibitory effects

α-amylase inhibitory activity of water and ethanol extracts for *U. davidiana* var. *japonica* was evaluated (Table 1). It was showed that that ethanol extracts had higher inhibitory activity than those of water extract. However, there was not significant difference between two groups (p>0.05). As a concentration of 1.0 mg/ml, the inhibitory activity of water and ethanol extracts of *U. davidiana* var. *japonica* were 40.7% and 41.9%, respectively. Although the α-amylase inhibitory activity of the bark was higher than root, there was not significant difference between two groups (p>0.05). Figure 1 was shown the rate of α-amylase inhibitory of Acarbose (positive control) and relative inhibitory rate for *U. davidiana* var. *japonica* on 1.0 M. The values of water extracts for root and bark were 38.6%, and 48.0%, respectively. The values for ethanol extracts for root and bark were 40.3% and 51.7%, respectively.

The IC_{50} value for α-amylase inhibitory activity were shown in Table 3. The IC_{50} for water extracts of root and bark were 22.89 and 17.96 ug/ml, respectively. They were significantly higher than that of Acarbose (9.41 ug/ml) (p<0.01). The mode of inhibition of the crude *U. davidiana* var. *japonica* extracts against α-amylase was confirmed by Lineweaver–Burk plots (Figure 3). Crude water extract of *U. davidiana* var. *japonica* inhibited α-amylase noncompetitively. Vmax decreases with uncompetitive inhibition (mixed inhibition). 1/Vmax was 25 and Km/Vmax was 1.667.

α-glucosidase inhibitory effects

The values of the α-glucosidase inhibitory effects for water and ethanol extracts of *U. davidiana* var. *japonica* were shown in Table 2. It was observed that inhibition percentage values go on increasing with enhancements in concentration of both extracts in the assay mixture. α-glucosidase inhibition of water extract for roots ranged from 24.9% at 0.25 mg/ml to 43.7% at 1.0 mg/ml. α-glucosidase inhibition of water extract for barks was ranged from 31.4% at 0.25 mg/ml to 51.6% at 1.0 mg/ml. However, there was not significant difference between two groups (F = 0.597, p>0.05). The values of α-glucosidase inhibition for ethanol extracts of roots and barks evaluated 45.1% at 1.0 mg/ml and 53.1%, respectively. The all values of α-glucosidase inhibitory for ethanol extracts were higher than those of water extracts. However, they are not showed a statistically significant difference (F= 0.288 for roots and 0.303 for barks, p<0.05).

Figure 2 was shown the rate of α-glucosidase inhibitory of Acarbose (positive control) and relative inhibitory rate for *U. davidiana* var. *japonica* extracts on 1.0 M. The values of water extracts for roots and barks were 44.1% and 58.9%, respectively. The values of ethanol extracts for roots and barks were 54.4% and 57.0%, respectively. The IC_{50} for α-glucosidase for water extracts of roots and barks were 18.34 and 16.05 ug/ml (Table 3). The IC_{50} for α-glucosidase for ethanol extracts of roots and barks were 17.22 and 14.57 ug/ml.

The mode of inhibition of the crude *U. davidiana* var. *japonica* extracts against α-glucosidase was confirmed by Lineweaver–Burk plots (Figure 4). Crude water extract mixed noncompetitively inhibited α-glucosidase. 1/Vmax was 32 and Km/Vmax was 2.133.

Table 1: The degree of α-amylase inhibition (%) of water and ethanol extracts of Ulmus davidiana var. japonica.

U. davidiana var. *japonica*	Concentration (mg/ml)	Solvent		*t*-test
		Water	Ethanol	
Root	0.25	21.89±0.44	23.01±1.56	0.261
	0.50	27.65±0.97	29.63±3.03	

	0.75	34.84±1.64	36.70±0.87	
	1.00	40.73±1.87	41.85±1.11	
Bark	0.25	27.25±2.37	29.55±2.74	0.533
	0.50	33.55±4.12	39.36±2.50	
	0.75	42.95±3.18	45.21±3.28	
	1.00	46.67±2.78	49.47±2.67	
F-test		0.006	0.597	
Data represented the mean ± SD from three replicates.				

Table 2: The degree of α-glucosidase inhibition (%) of water and ethanol extracts of *Ulmus davidiana* var. *japonica*.

U. davidiana* var. *japonica	**Concentration (mg/ml)**	**Solvent**		***t*-test**
		Water	**Ethanol**	
Root	0.25	24.89±0.89	26.63±0.99	0.288
	0.50	31.11±1.93	32.88±1.84	
	0.75	37.92±2.26	39.83±1.83	
	1.00	43.72±1.68	45.10±1.71	
Bark	0.25	31.37±2.56	33.45±2.13	0.303
	0.50	40.27±2.80	42.63±3.50	
	0.75	48.14±2.47	49.83±1.80	
	1.00	51.64±2.80	53.10±2.44	
F-test		0.597	0.596	
Data represented the mean ± SD from three replicates.				

Table 3: The 50% inhibition (IC_{50}) of α-amylase and α-glucosidase of *Ulmus davidiana* var. *japonica*.

Enzyme	**Sample**	**Solvent**	**IC_{50}**
α-amylase	Root	Water	22.89±1.23
		Ethanol	20.21±1.43
	Bark	Water	17.96±10.4
		Ethanol	15.31±1.52
	Acarbose	Water	9.41±0.93
		Ethanol	9.01±0.92
α-glucosidase	Root	Water	18.34±0.68
		Ethanol	17.22±1.21
	Bark	Water	16.05±0.70
		Ethanol	14.57±0.86
	Acarbose	Water	8.19±0.57
		Ethanol	8.32±0.68

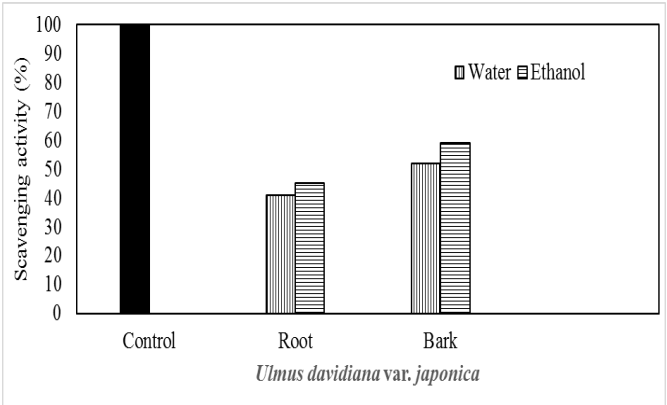

Figure 1: The rate of α-amylase inhibitory of Acarbose (Positive control) and Relative inhibitory rate for *Ulmus davidiana* var. *japonica* at different concentrations.

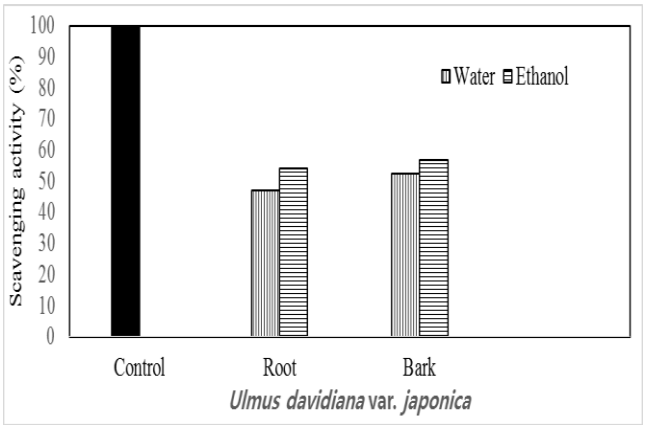

Figure 2: The rate of α-glucosidase inhibitory of Acarbose (Positive control) and Relative inhibitory rate for *Ulmus davidiana* var. *japonica* at different concentrations.

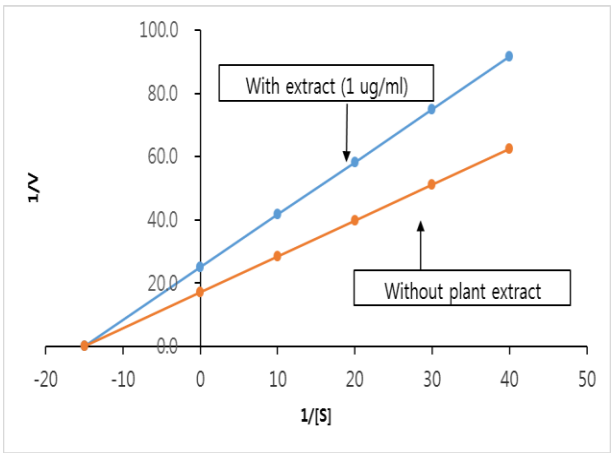

Figure 3: Lineweaver-Burk plot for the activity of α-amylase in the presence of concentration (1 ug/ml) of *Ulmus davidiana* var. *japonica* extract and inhibitor.

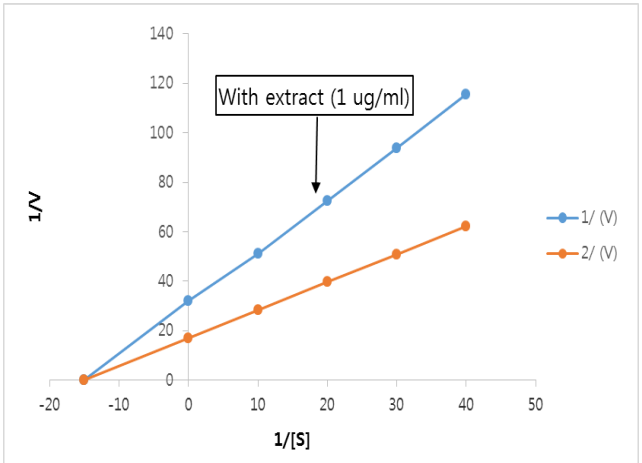

Figure 4: Lineweaver-Burk plot for the activity of α-glucosidase in the presence of concentration (1 ug/ml) of *Ulmus davidiana* var. *japonica* extract and inhibitor.

DISCUSSION

Sim and Han[8] estimated antioxidant activities, total phenolic and flavonoid contents of *Ulmus davidiana* root and bark extracts. Their results suggested that the ethanol extracts of *U. davidiana* root and bark, as well as their components, may be an alternative to more toxic synthetic antioxidants as additives in food, pharmaceutical, and cosmetic preparations. The DPPH radical scavenging activities of the *U. davidiana* root and bark extracts were IC_{50} 3.68 and 3.96 μg/mL, respectively.[8] Jung et al.[7] also reported that the DPPH radical scavenging activity (IC_{50}) of the methanol extract of *U. davidiana* bark was 2.81 μg/mL.

The *U. davidiana* root and bark extracts showed strong activities for NO.[8] Scavenging (IC_{50} 377.12 and 620.43

μg/mL respectively) and root and bark extracts showed strong activities for NO. scavenging (IC_{50} 377.12 and 620.43 μg/mL respectively).[8] The antioxidant function and digestion function were different, but the IC value was similar when compared.

The IC_{50} for water extracts of root and bark were ranged from 16.05 ug/ml to 22.89 ug/ml (Table 3). The IC_{50} for ethanol extracts of root and bark were ranged from 14.57 ug/ml to 20.21 ug/ml.

U. davidiana var. *japonica* was used for medicinal purposes in oriental medicine, and was presented in various functions. *U. davidiana* var. *japonica* might be used to develop potent anti-oxidant, anti-inflammatory, and anti-obesity agents, and may be useful as ingredients for related new functional raw materials.[17] It has high antioxidant properties. Cho et al.[18] thought that the possibility of development as a functional drink will be sufficient. Cancers [19] and physiological activity effects such as antibacterial effects, and anti-inflammatory properties of papillae have been reported.[20] However, The case report can suggest that ingestion of complementary medicine (elm bark root extracts) can be a cause of acute toxic hepatitis and acute kidney injury.[4] Research on elm trees has been actively conducted and their effects have been proven in some ways, but the effect of using elm trees is not the same for everyone, and indiscriminate folk remedies cannot consider individual sensitivity and can be a great health risk. In addition, an analysis of the ingredients representing side effects in elm-ironed water must be performed.[5]

Dereplication of secondary metabolites in bioactive extracts includes the determination of molecular mass and formula and cross-searching in the literature or structural natural products databases with taxonomic information, which greatly assists the identification process.[21] Therefore, screening of α-amylase and glucosidase inhibitors in natural plants has received much attention. Therefore, in the present study, he inhibitory effects of different extracts from *U. Davidian* var. *japonica* were shown against α-amylase and glucosidase enzyme activities. Elm trees have a 40-50% inhibitory effect on the small intestine when eating food, but they can exhibit digestive disorders. As a result, excess intake is banned.

Sim and Han[8] reported that the total phenolic and flavonoid contents of the *Ulmus davidiana* root extract were higher than those of the bark extract. Bong and Park[22] reported that phenol components extracted from the milky skin exhibited oxidase inhibitory effects related to ROS production. Therefore, the antioxidant activity of the papilla is expected to be due to phenol and flavonoid components.[17] However, Lim and Loh [23] reported that the α-glucosidase inhibition activity of bound phenolic extract has higher than free soluble phenolic extract because the bound phenolic mostly exists in a β-glycosides form that prefers aqueous phase than the free phenolic which is in the form of glycones, a non-sugar group. Thus, direct enzyme-inhibitor interaction is predicted to be higher in bound phenolic extracts in the α-glucosidase assay.[24]

CONCLUSIONS

The α-amylase and α-glucosidase inhibitory capacity of the bark of *U. davidiana* var. *japonica* was higher than those of roots. However, there was no significant difference between two groups (p>0.05). Although ethanol extracts had higher inhibitory activity than those of water extract, there was no significant difference between two groups. The results of this research showed that *U. davidiana* var. *japonica* bark represents can help to some extent in suppressing digestive enzymes.

REFERENCES

1. Makita H, Miyagi T, Miura O, Kikuchi T. A study of an alder forest and an elm forest with special reference to their geomorphological conditions in a small tributary basin. In: Vegetation und Lansdschaft Japans. Bull: Yokohama Phytosoc Soc Japan, 1979; 237-244.
2. Jun CD, Pae HO, Kim YC, Jeong SJ, Yoo JC, Lee EJ, Choi BM, Chae SW, Park RK, Chung HT. Inhibition of nitric oxide synthesis by n-butanol fraction of the methanol extract of *Ulmus davidiana* in murine macrophages. J Ethnophar-macol, 1998; 62: 129-35.
3. Guo JA, Wang MH. Antioxidant and antidiabetic activities of *Ulmus davidiana* extracts. J Food Sci Biotechnol, 2007; 16: 55-61.
4. Lee, SJ. Korean Folk Medicine, Monographs Series No. 3, Seoul National University Press, Seoul, Korea, 1996; 39.
5. Kim BH, Na SY, Sung JY, Chang JH, Kim S, Lee HH, Chung W, Jung JY. A case of acute toxic hepatitis and acute kidney injury after ingestion of *Ulmus davidiana* var. *japonica* extracts. The Korean Journal of Nephrology, 2010; 29: 136-39.
6. Kim JP, Kim WG, Koshino H, Jung J, Yoo ID. Sesquiterpene Onaphthoquinones from the root bark of *Ulmus davidiana*. Phytochem, 1996; 43: 425-30.
7. Jung HJ, Kang HJ, Song YS, Lim CJ, Park EH. Antioxidant and anti-nociceptive activities of *Ulmus davidiana* var. *japonica*. Biomolecules & Therapeuties, 2008; 16: 9-13.
8. Sim KH, Han YS. Antioxidant activity of ethanol extracts from the root and bark of *Ulmus davidiana*. Korean J Food Sci Technol, 2019; 51: 133-40.
9. Nielsen JE, Borchert, TV. Protein engineering of bacterial K-amylases. Biochimica et Biophysica Acta, 2000; 1543: 253-74.
10. Chen Y, Armstrong Z, Artola M, et al. Activity-based protein profiling of retaining α-amylases in complex biological samples. J Am Chem Soc, 2021; 143: 2423-32.
11. Bruni, CB. Sica V, Auricchio F, Covelli I. Further kinetic and structural characterization of the lysosomal α-D-glucoside glucohydrolase from cattle liver. Biochim Biophys Acta, 1970; 212: 470-7.

12. Mooradian AD, Thurman JE. Drug therapy of postprandial hyperglycaemia. Drugs, 1999; 57: 19-29.
13. Apostolidis E, Lee CM. In vitro potential of *Ascophyllum nodosum* phenolic antioxidant-mediated alpha-glucosidase and alpha-amylase. J Food Sci, 2010; 75: 97-102.
14. Deutschlander MS, van de Venter M, Roux S, Loux J, Lall N. Hypoglycaemic activity of four plant extracts traditionally used in South Africa for diabetes. J Ethnopharmacol, 2009; 124: 619-24.
15. Kim YM, Jeong YK, Wang MH, Lee WY, Rhee HI. Inhibitory effect of pine extract on alpha-glucosidase activity and postprandial hyperglycemia. Nutrition, 2005; 21: 756-61.
16. Lineweaver H, Burk D. The determination of enzyme dissociation constant. J Am Chem Soc, 1934; 56: 658-66.
17. Lee SG, Kang H. Anti-oxidant, anti-inflammatory, and anti-obesity effects of ethanol extracts from *Ulmus dividiana* var. *japonica*. Biomedical Science Letters, 2019; 25: 227-36.
18. Cho ML, Oh YN, Ma JG, Lee SJ, Choi YH, Son DH, Hee JE, Kim JY. Effect of extract temperature and duration on antioxidant activity and sensory characteristics of *Ulmus pumila* bark extract. Korean Journal of Food Preservation, 2016; 23: 995-1003.
19. Yang Y, Hyun JW, Lim KH, Kim HJ, Woo ER, Park J. Antineoplastic effect of extracts from traditional medical plants and various plants (III). Korea J Pharma, 1996; 27: 105-10.
20. Hong ND, Roh YS, Kim NJ, Kim JS. A study on efficacy of *Ulmi* cortex. Korean J Pharma, 1990; 1: 217-22.
21. Atanasov AG, Zotchev SB, Dirsch VM, The International Natural Product Sciences Taskforce, Supuran CT. Natural products in drug discovery: advances and opportunities. Nat Rev Drug Discov, 2021; 20: 200-16.
22. Bong JG, Park Y. Antioxidant effect of hederagenin 3-O-b-D-glucopyranosyl(1→3)-a-L-rhamnopyranosyl(1→2)-a-L-arabino pyranoside (HDL) isolated from root bark of *Ulmus davidiana*. J Life Sci, 2010; 20: 281-91.
23. Lim SM, Loh SP. In vitro antioxidant capacities and antidiabetic properties of phenolic extracts from selected citrus peels. Int Food Res J, 2016; 23: 211-9.
24. Widyawati PS, Werdani YW, SetiokusumoC, Kartikasari A. In vitro antioxidant capacities and antidiabetic properties of pluchea leaves and green tea mixtures at various proportions. International Journal of Pharmacy and Pharmaceutical Sciences, 2017; 9: 203-8.