

# Inhibitory effects of aqueous extract of *Eremurus spectabilis* M. Bieb. on diabetes mellitus and skin related enzymes

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**Cite this article as:** Bayrak, B. B., & Yanardağ, R. (2021). Inhibitory effects of aqueous extract of *Eremurus spectabilis* M. Bieb. on diabetes mellitus and skin related enzymes. *Istanbul Journal of Pharmacy*, 51(3), 357-364.

## ABSTRACT

**Background and Aims:** Nutritional bioactive and natural ingredients isolated from wild edible plants, with promising effects against diabetes and/or diabetes-related diseases continue to be investigated. There is increasing interest in these substances due to their protective/therapeutic potential. The purpose of the present study is to investigate the inhibitory potential of aqueous extract of *Eremurus spectabilis* M. Bieb. on diabetes mellitus and skin-related enzymes.

**Methods:** Fresh *E. spectabilis* leaves were obtained from a local market in Eyup/İstanbul, Turkey and their aqueous extract was prepared. Inhibitory activities of the aqueous extract of this plant on  $\alpha$ -amylase,  $\alpha$ -glucosidase, elastase, hyaluronidase, and tyrosinase were examined.

**Results:** The aqueous extract of *E. spectabilis* showed effective inhibitor activity against both  $\alpha$ -amylase and  $\alpha$ -glucosidase as compared to acarbose. On the other hand, the aqueous extract exhibited a moderate inhibitory activity on elastase. Whereas, it exhibited a weak inhibitory effect on the activities of hyaluronidase and tyrosinase when compared to their respective standard inhibitors.

**Conclusion:** The current findings suggest that the consumption of *E. spectabilis* leaves may be of benefit for regulation of postprandial blood glucose, as shown by the strongly inhibiting action of its extract on both  $\alpha$ -amylase and  $\alpha$ -glucosidase. In addition, it might be beneficial against skin-related disorders, because of its inhibitory action on elastase activity.

**Keywords:** *Eremurus spectabilis*, Inhibitory activity, Diabetes mellitus, Skin-related enzymes

## INTRODUCTION

Plants are an indispensable food source, due to their nutritional and medicinal properties, as well as being the primary sources of biochemically active phytochemicals. Besides, they are used as an alternative treatment option to modern medicine. Therefore, investigation of various plants and plant product-based inhibitors from nature have been of great interest to researchers. From this point of view, screening natural sources-based potent inhibitors of enzymes that play key roles in diabetes and many other diseases have attracted immense attention, due to the toxic and/or adverse/unwanted effects of chemically synthesized compounds (Li & Ma, 2017; Marrelli, Statti, & Conforti, 2020). The genus *Eremurus* (an important genus of *Xanthorrhoeaceae* that comprises approximately 60 species) is widespread in Central Asia and Middle East (including Turkey) (Beiranvand & Beiranvand, 2021; Salehi et al., 2017). *E. spectabilis* is naturally grown in Eastern and Southeastern Anatolia regions of Turkey. It is popularly referred to as "Çiriş otu" in these regions (Aysu, Demirbas, Bengü, & Küçük, 2015; Cinar et al., 2017; Tosun et al., 2012). Besides its nutritional features, the *Eremurus* species also possesses medicinal potential. The leaves and roots are traditionally used to cure diseases

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Submitted: 07.05.2021

Revision Requested: 31.05.2021

Last Revision Received: 02.06.2021

Accepted: 03.06.2021

Published Online: 00.00.0000

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such as hemorrhoids, eye inflammation, jaundice, fungal infection, hypertension, and eczema (Dashti, Tavakoli, Zarif Ketabi, & Paryab, 2005; Gaggeri et al., 2013; Gaggeri et al., 2015). In addition, this wild edible plant is used to manage or cure gastrointestinal and liver disorders (Vala, Asgarpanah, Hedayati, Shirali, & Bejestani, 2011). Owing to the aforementioned traditional use of this plant, the phenolic compounds (major secondary metabolites) in some *Eremurus* species were isolated and their biological activities were investigated (Abubaker & Hidayat, 2015; Karaoglan Esen et al., 2018; Mottaghipisheh et al., 2021).

Diabetes mellitus (DM) is an endemic multifactorial endocrine disease characterized by insulin deficiency or resistance, which gives rise directly to hyperglycemia (Bonamonte & Filoni, 2021). The most important underlying factor in the development of DM is the deterioration of the regulatory effect of pancreatic  $\beta$ -cells on glucose metabolism, thus disrupting the balance between insulin production and secretion (Zafar et al., 2021). Coupled to this, the hydrolytic activities of two catabolic enzymes (e.g.  $\alpha$ -amylase and  $\alpha$ -glucosidase) on carbohydrate polymers (such as glycogen and starch) give rise to elevated blood glucose levels.

$\alpha$ -Amylase is an enzyme responsible for the conversion of complex carbohydrates (e.g. starch) into simple oligosaccharides (Pant et al., 2021).  $\alpha$ -Glucosidase on the other hand is a hydrolytic enzyme catalyzing the cleavage of  $\alpha$ -glycosidic bonds of both endogenous and exogenous carbohydrate polymers. The inhibition of these aforementioned enzymes is as important as dietary restriction in controlling hyperglycemia (Lankatillake et al., 2021; Papoutsis et al., 2021).

Skin disorders have been associated with prevalent complications of DM. Also, hyperglycemia leads to deterioration of cells in the dermis layer of skin. Thus, altering the flexibility and solubility of collagen as a consequence of altered advanced glycation end products (de Maceto, Nunes, & Barreto, 2016; Lai, Nor, Kamaruddin, Jamil, & Safian, 2021). Accumulated evidence shows that skin diseases such as sclerodema, diabetic dermopathy, necrobiosis lipoidica, and acanthosis nigricans are strongly associated with DM (Demirkesen 2015; Mendes et al., 2017; Svoboda & Shields, 2021). For this reason, natural compounds that can inhibit  $\alpha$ -amylase and  $\alpha$ -glucosidase, and as well regulate postprandial glucose may have great significance in managing DM and DM-related skin disorders.

Elastases are proteolytic enzymes that mainly hydrolyze proteins of the connective tissue in lungs, arteries, ligaments, and skin. These enzymes also participate in a controlled proteolysis of elastic fibers during normal growth and tissue remodeling. However, under certain pathological conditions, the activity of these enzymes can lead to both major and uncontrolled destruction of structural proteins, thereby triggering severe diseases like pulmonary emphysema, acute pancreatitis, rheumatoid arthritis, thrombosis, stroke, and skin-aging (Desmiaty et al., 2020). To delay aging, inhibitors of elastase enzymes can be useful tools via preventing loss of skin elasticity and skin sagging (Apraj & Pandita, 2016).

The hydrolysis of glycosaminoglycans such as hyaluronic acid (HA) of the extracellular matrix (ECM) especially of the skin and other connective tissues is carried out by hyaluronidases. They play a crucial role in controlling the size and concentration of HA chains. Elevated hyaluronidase activity causes tissue injury, due to an increase in decomposition of HA (Buhren et al., 2020). Altered activities of these enzymes are implicated in cases of cancer, liver diseases, allergy, wound healing, and dermatological disorders, including skin-wrinkling and skin-aging (Gangadharan, Jacob, & Densely Jose, 2014; Girish & Kemparaju, 2007).

Tyrosinase, a multifunctional membrane oxidase (or oxidoreductase), catalyzes the first step of both the catecholamine and melanin biosynthesis (Pillaiyar, Manickam, & Navasivayam, 2017). Melanin accumulation may cause serious skin diseases such as melasma, freckles, senile lentigo, age spots, and sites of actinic damage (Zolghadri et al., 2019). Therefore, many tyrosinase inhibitors find application in cosmetics and pharmaceutical products, for preventing the overproduction of melanin in the epidermis (Ullah et al., 2019).

Inhibitions of these enzymes mentioned above have gained great importance in DM and DM-associated skin diseases for many years. There are insufficient published reports about the effects of *E. spectabilis* on inhibitory effects of these enzymes. Therefore, the objective of the present study was to investigate the inhibition activities of aqueous extract of *E. spectabilis* on the  $\alpha$ -amylase,  $\alpha$ -glucosidase, elastase, hyaluronidase, and tyrosinase.

## MATERIALS AND METHODS

### Plant material and chemicals

Fresh *E. spectabilis* leaves were obtained from a local market in Eyup/Istanbul, Turkey. The plant was inspected and identified by Prof. Dr. Emine Akalin (Faculty of Pharmacy, İstanbul University). A specimen was deposited at the Faculty of Pharmacy Herbarium of İstanbul University (ISTE 93132). The plant material was washed twice with distilled water, and thereafter dried at room temperature. The dried plant was stored in  $-20^{\circ}\text{C}$  until they were required for use.

The enzymes and their respective substrates (i.e.,  $\alpha$ -amylase,  $\alpha$ -glucosidase, elastase, hyaluronidase, and tyrosinase; soluble starch, *p*-nitrophenyl- $\alpha$ -D-glucopyranoside ( $\alpha$ -PNPG), N-Succinyl-Ala-Ala-Ala-*p*-nitroanilide (STANA), hyaluronic acid sodium salt, and L-tyrosine) were purchased from Sigma-Aldrich (St. Louis, MO, USA) or Merck (KGaA, Darmstadt, Germany). All chemicals used in the experiment were of analytical grade.

### Preparation of extract

Aqueous extract was prepared by refluxing 20g of dried *E. spectabilis* leaves in 200mL of distilled water for 6 hours in reflux condenser. The extract was then filtered at room temperature and lyophilized to dryness. The resultant lyophilized aqueous extract of the plant was stored in deep freeze (at  $-20^{\circ}\text{C}$ ) until use.

### In vitro $\alpha$ -amylase inhibitory activity

$\alpha$ -Amylase inhibitory activity was examined according to Al-Dabbas et al., (Al-Dabbas, Kitahara, Suganuma, Hasimato, &

Tadera, 2006). In brief, a soluble starch solution (substrate, in 0.25 M phosphate buffer, pH 7.0) was mixed with varying concentrations of the extract, then the mixture was allowed to stand at 37°C for 5 min. Equal aliquots of  $\alpha$ -amylase solution and phosphate buffer (0.25 M pH 7.0) were added to the reaction medium and then further incubated at 37°C for 7.5 min. Appropriate aliquots of 0.01 N iodine solution and distilled water were pipetted into the solution, then the absorbance of resulting mixture was recorded at 660nm by using a spectrophotometer. Acarbose was used as a standard inhibitor. Results were expressed as the average of triplicate trials.

Percentage inhibition was determined using the following formula:

$$\alpha\text{-Amylase inhibitory activity (\%)} = \left[ \frac{(A_0 - A_1)}{A_0} \right] \times 100$$

$A_0$  is the enzyme activity without inhibitor.

$A_1$  is the activity in presence of inhibitor.

#### **In vitro $\alpha$ -glucosidase inhibitory activity**

$\alpha$ -Glucosidase inhibitory activity was assessed by the method of Tao et al., with a slight modification using  $\alpha$ -PNPG as a substrate (Tao, Zhang, Cheng, & Wang, 2013). Briefly, a phosphate buffer (0.1 M, pH 7.40), appropriate aliquot of different concentrations of the extract, and  $\alpha$ -glucosidase (in phosphate buffer pH 7.40) were mixed in 96-well plate. After pre-incubation (at 37°C for 10 min),  $\alpha$ -PNPG (in phosphate buffer pH 7.40) was pipetted into the reaction mixture, and then incubated at 37°C for another 10 min. At the end of the incubation period, the absorbance was recorded at 410nm by using a microplate reader. Acarbose was used as the standard inhibitor instead of the plant extract. Results were expressed as the average of triplicate trials.

Percentage inhibition was calculated using the following formula:

$$\alpha\text{-Glucosidase inhibitory activity (\%)} = \left[ \frac{(A_0 - A_1)}{A_0} \right] \times 100$$

$A_0$  is the enzyme activity without inhibitor.

$A_1$  is the activity in presence of inhibitor.

#### **In vitro elastase inhibitory activity**

Elastase inhibitory activity was determined by spectrophotometric method of Moon et al., (Moon, Yim, Song, Lee, & Hyun, 2010). The assay mixture, containing enzyme solution, plant extract (or standard inhibitor) and Tris-HCl buffer (200 mM, pH 7.8), was pre-incubated at 37°C for 15 min. The reaction was initiated by adding a substrate (STANA) solution to the test tube and the mixture was incubated at the same temperature for 30 min. At the end of the incubation period, the absorbance was measured at 410nm. Ursolic acid was used as the standard compound instead of the plant extract. Results were expressed as the average of triplicate trials.

The percentage of elastase inhibitory activity was determined according to the following equation:

$$\text{Elastase inhibitory activity (\%)} = \left[ \frac{(A_0 - A_1)}{A_0} \right] \times 100$$

$A_0$  is the enzyme activity without inhibitor.

$A_1$  is the activity in presence of inhibitor.

#### **In vitro hyaluronidase inhibitory activity**

Hyaluronidase inhibitory activity was assayed by the spectrophotometric method developed by Lee et al., in which sodium hyaluronate is formed from *N*-acetylglucosamine (Lee, Kim, Cho, & Choi, 1999). The reaction mixture, containing suitable aliquot of bovine testes hyaluronidase dissolved in 0.1 M acetate buffer (pH 4.0) was mixed with different concentrations of the extract, and then incubated in a water bath at 37°C for 20 min. For the control experiment, solvent from which the extracts were dissolved was used. The absorbance of the reaction mixture was then measured by using the spectrophotometer at 585nm. Rutin was used as the standard inhibitor instead of the plant extract. Results were expressed as the average of triplicate trials.

The percent inhibition of hyaluronidase was calculated using the following equation:

$$\text{Hyaluronidase inhibitory activity (\%)} = \left[ \frac{(A_0 - A_1)}{A_0} \right] \times 100$$

$A_0$  is the enzyme activity without inhibitor.

$A_1$  is the activity in presence of inhibitor.

#### **In vitro tyrosinase inhibitory activity**

Tyrosinase inhibitory activity was determined according to the procedure described by Vanni et al., (Vanni, Gastaldi, & Giunata, 1990). Briefly, tyrosinase solution, L-tyrosine, and different concentrations of the extract were added to a solution of phosphate buffer (0.1 M, pH 6.5). The test mixture was incubated for 10 min at 37°C and the absorbance was monitored at 475nm. Kojic acid was used as the standard inhibitor instead of the plant extract. Results were expressed as the average of triplicates.

The percent inhibition of tyrosinase was determined according to the following equation:

$$\text{Tyrosinase inhibitory activity (\%)} = \left[ \frac{(A_0 - A_1)}{A_0} \right] \times 100$$

$A_0$  is the enzyme activity without inhibitor.

$A_1$  is the activity in presence of inhibitor.

For  $\alpha$ -amylase,  $\alpha$ -glucosidase, elastase, hyaluronidase, and tyrosinase inhibitory activities, the extract (or standard) concentration resulting in a 50% inhibition ( $IC_{50}$ ) was calculated by regression equations (by plotting the extract solution concentration versus percentage of inhibition). Lower  $IC_{50}$  values indicate higher inhibitory potential of the tested plant extract.

## **RESULTS**

#### **In vitro $\alpha$ -amylase inhibitory activity of extract**

The inhibition effects of aqueous extract of *E. spectabilis* as well as that of acarbose against  $\alpha$ -amylase activity are summarized in Table 1. According to the results, both the aqueous extract and the standard inhibitor exerted an  $\alpha$ -amylase inhibition in a dose-dependent manner with  $IC_{50}$  values of  $0.043 \pm 0.003 \mu\text{g/mL}$  and  $91.84 \pm 0.94 \mu\text{g/mL}$ , respectively. Considering the high

inhibitory activities (associated with the lower  $IC_{50}$  values), the aqueous extract demonstrated a higher inhibitory activity against  $\alpha$ -amylase in comparison to acarbose. The order for an inhibitory effect is as follows: aqueous extract > acarbose (Table 1).

#### **In vitro $\alpha$ -glucosidase inhibitory activity of extract**

The inhibitory activities and  $IC_{50}$  values of an aqueous extract and acarbose on  $\alpha$ -glucosidase are given in Table 2. It was found that  $\alpha$ -glucosidase inhibition increased in a concentration-dependent manner for all tested samples. Accord-

ing to the results, the  $IC_{50}$  values of aqueous extract and the acarbose were calculated as  $1.27 \times 10^{-3} \pm 7.78 \times 10^{-5}$   $\mu\text{g/mL}$  and  $98.71 \pm 1.13$   $\mu\text{g/mL}$ , respectively. As a result, the aqueous extract had a much lower  $IC_{50}$  value than that of the acarbose. The  $\alpha$ -glucosidase inhibitory activity of *E. spectabilis* extracts and the standard decreased in the order of: aqueous extract > acarbose (Table 2).

#### **In vitro elastase inhibitory activity of extract**

The inhibitory activities of aqueous extract of *E. spectabilis* and ursolic acid against elastase are shown in Table 3. The elastase

**Table 1.  $\alpha$ -Amylase inhibitory activity of *E. spectabilis* extract.**

Extract/Standard	Concentrations ( $\mu\text{g/mL}$ )	Inhibition (%)*	$IC_{50}$ ( $\mu\text{g/mL}$ )*
Aqueous Extract	0.0001	17.39 $\pm$ 2.90	0.043 $\pm$ 0.003
	0.001	25.99 $\pm$ 2.25	
	0.005	33.31 $\pm$ 2.42	
	0.01	45.51 $\pm$ 2.29	
	0.05	51.19 $\pm$ 2.06	
Acarbose	0.1	19.71 $\pm$ 1.26	91.84 $\pm$ 0.94
	1	32.44 $\pm$ 1.73	
	10	43.37 $\pm$ 2.01	
	100	54.44 $\pm$ 0.56	
	1000	64.32 $\pm$ 2.80	

\*Mean $\pm$ SD, Results were expressed as the average of triplicate trials.

**Table 2.  $\alpha$ -Glucosidase inhibitory activity of *E. spectabilis* extract.**

Extract/Standard	Concentrations ( $\mu\text{g/mL}$ )	Inhibition (%)*	$IC_{50}$ ( $\mu\text{g/mL}$ )*
Aqueous Extract	$5 \times 10^{-5}$	4.91 $\pm$ 0.66	$1.27 \times 10^{-3} \pm 7.78 \times 10^{-5}$
	$1 \times 10^{-4}$	7.65 $\pm$ 0.96	
	$2 \times 10^{-4}$	13.37 $\pm$ 1.54	
	$4 \times 10^{-4}$	21.11 $\pm$ 0.80	
	$6 \times 10^{-4}$	23.68 $\pm$ 1.44	
Acarbose	0.1	16.58 $\pm$ 1.12	98.71 $\pm$ 1.13
	1	31.91 $\pm$ 1.78	
	10	39.26 $\pm$ 0.73	
	100	50.67 $\pm$ 0.59	
	1000	63.78 $\pm$ 1.38	

\*Mean $\pm$ SD, Results were expressed as the average of triplicate trials.

**Table 3. Elastase inhibitory activity of *E. spectabilis* extract.**

Extract/Standard	Concentrations ( $\mu\text{g/mL}$ )	Inhibition (%)*	$IC_{50}$ ( $\mu\text{g/mL}$ )*
Aqueous Extract	0.01	14.83 $\pm$ 1.32	60.30 $\pm$ 2.00
	0.1	18.00 $\pm$ 1.15	
	0.5	21.70 $\pm$ 1.75	
	1	34.28 $\pm$ 2.92	
	50	41.48 $\pm$ 1.36	
Ursolic Acid	0.01	21.09 $\pm$ 3.86	38.58 $\pm$ 3.29
	0.1	35.01 $\pm$ 0.38	
	1	39.92 $\pm$ 1.88	
	10	48.21 $\pm$ 3.15	
	100	73.40 $\pm$ 1.86	

\*Mean $\pm$ SD, Results were expressed as the average of triplicate trials.

inhibition by the aqueous extract and the standard were exhibited in a dose-dependent manner. At 1 µg/mL concentration, it was observed that both aqueous extract of *E. spectabilis* and ursolic acid had very close inhibition values (34.28±2.92% and 39.92±1.88%, respectively). IC<sub>50</sub> values of aqueous extract and ursolic acid were diminished in the following order: Ursolic acid (38.58±3.29 µg/mL) > aqueous extract (60.30±2.00 µg/mL) (Table 3).

#### **In vitro hyaluronidase inhibitory activity of extract**

In Table 4, the aqueous extract of *E. spectabilis* and rutin are depicted. A comparison shows that the aqueous extract had a lower inhibition effect than that of rutin (standard inhibitor). Whereas, rutin had the lower IC<sub>50</sub> value (0.68±0.01 µg/mL) in comparison to the aqueous extract (2517.50±59.09 µg/mL) of *E. spectabilis* (Table 4).

#### **In vitro tyrosinase inhibitory activity of extract**

In this study, the inhibitory activities of aqueous extract of *E. spectabilis* and kojic acid on tyrosinase are presented in Table 5. The aqueous extract and kojic acid exhibited tyrosinase inhibitor activities in a dose-dependent manner. The IC<sub>50</sub> values of *E. spectabilis* and kojic acid increased in the order of: Kojic acid (74.14±4.76 µg/mL) > aqueous extract (9391.02±180.54 µg/mL) (Table 5).

## **DISCUSSION**

Diabetes mellitus is multifactorial and the most prevalent endocrine disorder and, is increasing in prevalence. Not only multiorgan (e.g. eyes, kidneys, and skin). but also blood vessels are unavoidably affected. Moreover, DM leads to an enormous financial burden and a low quality of life. Long-term detrimental effects of DM may impair skin homeostasis resulting in skin diseases such as infections, diabetic foot syndrome, lichen planus, pruritus, and vitiligo in at least one-third of all diabetics (Behm, Schreml, Landthaler, & Babilas, 2012).

Since plants consumed as food are easily accessible and contain natural ingredients, their effects against DM and DM-related diseases are constantly being investigated. For an antidiabetic effect, the focus is mostly on the inhibition of α-amylase and α-glucosidase- the enzymes associated with digestion of carbohydrates. Therefore, inhibitors of these two catabolic enzymes can decrease the release of D-glucose from dietary carbohydrates, slow down glucose absorption, lower blood glucose levels and reduces hyperglycemia (Etsassala et al., 2019; Gong et al., 2020). In this study, both α-amylase and α-glucosidase inhibitory activities of aqueous extract of *E. spectabilis* were found to be remarkable lower (IC<sub>50</sub> values) than that of acarbose. It has been reported that *E. persicus* extracts had less than 10% in-

**Table 4. Hyaluronidase inhibitory activity of *E. spectabilis* extract.**

Extract/Standard	Concentrations (µg/mL)	Inhibition (%)*	IC <sub>50</sub> (µg/mL)*
Aqueous Extract	750	22.17±2.92	2517.50±59.09
	1000	30.44±1.00	
	2000	52.87±2.43	
	4000	70.46±1.40	
	5000	75.98±2.31	
Rutin	0.001	16.94±1.33	0.68±0.01
	0.01	32.72±1.53	
	0.1	45.04±2.03	
	1	72.46±1.03	
	5	78.44±1.52	

\*Mean±SD, Results were expressed as the average of triplicate trials.

**Table 5. Tyrosinase inhibitory activity of *E. spectabilis* extract.**

Extract/Standard	Concentrations (µg/mL)	Inhibition (%)*	IC <sub>50</sub> (µg/mL)*
Aqueous Extract	100	6.17±2.14	9391.02±180.54
	1000	12.45±2.45	
	5000	39.20±2.45	
	10000	59.03±0.47	
	15000	66.46±1.27	
Kojic Acid	25	13.28±2.01	74.14±4.76
	50	42.21±5.27	
	75	50.74±3.33	
	100	80.87±2.34	
	250	89.26±1.32	

\*Mean±SD, Results were expressed as the average of triplicate trials.

hibitory activity on  $\alpha$ -glucosidase enzyme *in vitro* (Gholamhoseinian, Hossein, Fariba, & Mirtaj Aldini, 2008). It has been shown that ethyl acetate, methanol, and aqueous extracts of *E. himalaicus* (at a dose of 500 mg/kg body weight) administered to alloxan-induced diabetic rats provided a significant decrease in fasting blood glucose levels compared to diabetic rats. These effects have been suggested to be a mechanism independent on insulin secretion or inhibition of endogenous glucose production (Tramboo, 2013). Sacan, Akev, & Yanardag (2017) revealed that *Aloe vera* (L.) Burm. f. extracts were potent *in vitro*  $\alpha$ -amylase inhibitors. In addition, it was demonstrated that administration of *Aloe vera* (L.) Burm. f. leaf extract to diabetic rats led to a decrease in blood glucose levels (Okyar, Can, Akev, Baktir, & Sutlupinar, 2001). Meanwhile, the inhibitory effects of *E. spectabilis* against both  $\alpha$ -amylase and  $\alpha$ -glucosidase may be attributed to its richness in varied amount of active constituents and antinutritional factors, which are reported to include sterols such as  $\beta$ -sitosterol and ergosterol; flavonoids (e.g. rutin, morin, and quercetin) and resveratrol (Bircan & Kirbağ, 2015).

Elastase and hyaluronidase are the group of dermal enzymes belonging to matrix metalloproteinases (MMPs). They are known to be ECM hydrolyzing enzymes (Genc, Guragac Dereli, Saracoglu, & Kupeli Akkol, 2020). It has been shown that the elevation of these enzyme activities causes degradation of ECM which leads to loss of skin integrity (Acikara, Ilhan, Kurtul, Šmejkal, & Küpeli Akkol, 2019). Uncontrolled and excessive hydrolysis of these proteins brings about an increase in wrinkle formation, and eventual skin aging (Buhren et al., 2020; Deniz et al., 2020). On the other hand, tyrosinase, a melanogenic enzyme, participates in melanogenesis (i.e. formation of melanin, which is one of the basic constituents of hair, eye, and skin colour). Additionally, elevated melanin levels and its uncontrolled synthesis can give rise to skin disorders such as acanthosis nigricans, melasma, and senile lentiginos (Zolghadri et al., 2019). Hence, the search for new inhibitors of these enzymes so as to preserve the natural structure and maintain a healthy skin continues. In a comprehensive study by Chiocchio et al., (2018), elastase inhibitory activities of a hundred plants were screened and eleven of these plants were shown to strongly inhibit elastase activities. Celik Onar, Yusufoglu, Turker, & Yanardag (2012) revealed that the aqueous extract of *Epilobium angustifolium* L. leaves exerted strong antielastase activity with a low  $IC_{50}$  value. It was also reported that the total phenolic and flavonoid contents of the plants were positively correlated with their elastase inhibitory activities. In our study, the aqueous extract of *E. spectabilis* had moderate inhibitory activity against elastase as compared to ursolic acid. These effects of *E. spectabilis* may be attributed to its richness in varied amounts of phytochemical factors, which possibly possess inhibitory properties (Kumud & Sanju, 2018). Moreover, the number of hydroxyl groups of phenolics found in *E. spectabilis* tends to form stronger hydrogen bonds with the enzyme's functional side chain groups. This may have led to the higher inhibitory activity of the extracts on the enzymes (Wittenauer et al., 2015). However, the aqueous extract of *E. spectabilis* had a very weak inhibitory effect against both hyaluronidase and tyrosinase enzymes when compared with

their respective standard inhibitors (rutin and kojic acid). The possible explanation for the low inhibitory activities of this plant extract might be associated with secondary metabolites found in *E. spectabilis* which are poorly interacting with the active site of both enzymes.

## CONCLUSION

Considering the outcomes, the aqueous extract of *E. spectabilis* clearly exhibited inhibitory activities against both  $\alpha$ -amylase and  $\alpha$ -glucosidase enzymes. Moreover, the potent inhibitory effect of this herb on these catabolic enzymes in a carbohydrate metabolism may be promising for diabetics in the regulation of postprandial blood sugar. On the other hand, the extract of *E. spectabilis* showed a better inhibitor effect on elastase than that of both hyaluronidase and tyrosinase. More so, it could be suggested that this plant might be of benefit for usage as a raw extract form for skin-related disorders, because of its good inhibitory action on elastase activity. Thus, more supporting researches against antidiabetic effects of *E. spectabilis* both *in vitro* and *in vivo* should be conducted.

**Peer-review:** Externally peer-reviewed.

**Author Contributions:** Conception/Design of Study- B.B.B., R.Y.; Data Acquisition-B.B.B.; Data Analysis/Interpretation- B.B.B., R.Y.; Drafting Manuscript- B.B.B., R.Y.; Critical Revision of Manuscript- B.B.B., R.Y.; Final Approval and Accountability- B.B.B., R.Y.

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

**Financial Disclosure:** This study was supported by İstanbul University-Cerrahpaşa Scientific Research Projects Units with the grant number 52245.

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