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Original Article

In vitro inhibitory potential of *Amaranthus lividus* L. against therapeutic target enzymes

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ABSTRACT

Background and Aims: The search for new enzyme inhibitors in plants is attractive because they can be used as drugs in the treatment of various diseases. *Amaranthus* spp. (Amaranthaceae) includes about 70 different species, some of which are edible and some of which are used in traditional medicine to treat various ailments. *Amaranthus lividus* L. is a vegetable whose stems and leaves are used for human consumption in Turkey.
Methods: In this study, the *in vitro* enzyme inhibition potential of *A. lividus* on α-amylase, α-glucosidase, acetylcholinesterase (AChE), elastase, lipase, neuraminidase and tyrosinase was investigated for the first time. For this purpose, water extract of *A. lividus* was prepared. The tests of enzyme inhibitory activity were carried out by spectrophotometric and fluorometric methods.
Results: The water extract showed significant α-glucosidase and α-amylase inhibitory activities. Also, it displayed good elastase, lipase and tyrosinase inhibitory activities. However, it exhibited low inhibitory activity on AChE and neuraminidase.
Conclusion: The plant and its active constituents may be used as an antidiabetic enzyme inhibitor with future phytochemical constituent analysis.

Keywords: α-Amylase, α-Glucosidase, Acetylcholinesterase, Elastase, Lipase, Tyrosinase

INTRODUCTION

Enzymes continue to be important targets for drug development because altering enzyme activity has led to immediate therapeutic effects (Ramsay & Tipton, 2017). The search for natural enzyme inhibitors in plants is one of many investigations in the discovery and development of new drugs. Many compounds isolated from medicinal plants have the potential to inhibit enzymes and these compounds are used as therapeutic agents (Rauf & Jehan, 2017).

Diabetes mellitus is a chronic disease characterized by high blood glucose levels. Type 2 diabetes mellitus is the most common form of diabetes (more than 90% of all cases). One of the most widely used classes of oral antidiabetic drugs for the treatment of type 2 diabetes mellitus are the α-glucosidase inhibitors (Şöhretoğlu & Sari, 2020). α-Glucosidase (EC 3.2.1.20) is responsible for catalyzing the hydrolysis of disaccharides (maltose and sucrose). One of the other enzymes responsible for catalyzing starch hydrolysis is α-amylase (EC 3.2.1.1). The function of α-amylase is to catalyze the hydrolysis of α-(1→4)-glycosidic bonds of starch (Papoutsis et al., 2021). α-Glucosidase inhibitors reduce hyperglycemia by delaying glucose absorption and lowering postprandial blood glucose and insulin levels (Şöhretoğlu & Sari, 2020). Miglitol, acarbose, and voglibose are some of the clinically used

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α-glucosidase and α-amylase inhibitors (Papoutsis et al., 2021). There is a need to find new antidiabetic agents as the efficacy of therapy mainly depends on the patients (Şöhretoğlu & Sari, 2020).

Alzheimer's disease is one of the major causes of dementia and is characterized by a progressive neurodegenerative disorder. Cholinergic dysfunction has been closely associated with early cognitive decline in Alzheimer's disease patients. Most of the drugs currently used to treat dementia are acetylcholinesterase (AChE; EC 3.1.1.7) inhibitors, namely donepezil, rivastigmine, and galantamine. AChE inhibitors increase synaptic acetylcholine levels and improve cholinergic function in the brain (Santos, Gomes, Pinto, Camara, & Paes, 2018).

Elastase (EC 3.4.4.7) is responsible for the degradation of elastin and other proteins such as collagen and fibronectin (which are fundamental for the elasticity of the extracellular matrix). Excessive hydrolysis of elastin leads to loss of skin elasticity and subsequently skin aging. Elastase inhibitors are used as skin lighteners, anti-aging and anti-wrinkle agents. They are also used in the treatment of dermatological diseases and to promote the maintenance of skin elasticity (Chiocchio et al., 2018).

Inhibitors of lipase (EC 3.1.1.3) play a critical role in human lipid metabolism. A major cause of obesity is often excessive fat intake/absorption and accumulation. Obesity and hyperlipidemia are associated with a number of risk factors such as insulin resistance, impaired glucose tolerance, and hypertension, which may increase mortality rates. Lipase inhibitors have lipid-lowering effects by reducing the digestion and absorption of lipids from food and controlling the fat that enters the blood. Lipase inhibitors such as orlistat have become important in the treatment of obesity (Liu, Liu, Chen, & Shi, 2020).

Influenza is an infectious disease that leads to thousands of deaths and is caused by the influenza virus (Mahal et al., 2021). Neuraminidase (EC 3.2.1.18) is a membrane-bound glycoprotein, playing an important role in the viral life cycle (Wang et al., 2017). Neuraminidase inhibitors have potency against influenza A and B viruses responsible for the annual influenza epidemics. These inhibitors prevent the cleavage of sialic acid by viral neuraminidase, thereby inhibiting cell interactions between virus-host (Mahal et al., 2021). These inhibitors, including oseltamivir, zanamivir, and peramivir, are the main class of antivirals available for clinical use. However, the development of resistance to neuraminidase inhibitors is a public health concern (Lee & Hurt, 2018).

Melanin is produced by melanogenesis which is a major protective factor against ultraviolet radiation damage and it is the main pigment responsible for the pigmentation of skin, hair and eyes in humans (Zolghadri et al., 2019). However, uncontrolled and excessive melanogenesis may cause skin disorders, including freckles, age spots and hyperpigmentation (Chatatikun, Yamauchi, Yamasaki, Aiba, & Chiabchalard, 2019). Tyrosinase (EC 1.14.18.1) is a multifunctional metalloenzyme that acts as a rate-limiting enzyme in the synthesis of melanin. Therefore, inhibition of tyrosinase activity is essential for the treatment of pigmentation disorders in mammals (Zolghadri et al., 2019). Tyrosinase inhibitors are used in medical and cosmetic products such as skin lighteners, anti-aging and anti-wrinkle agents, and in the treatment of dermatological diseases (Chiocchio et al., 2018).

Many natural compounds obtained from plants are used in the treatment of various diseases due to their inhibitory effects on various enzymes. Therefore, numerous plants are still being studied to identify effective enzyme inhibitors and to use them in medical and cosmetic products (Zolghadri et al., 2019).

The genus *Amaranthus* (Amaranthaceae) includes 70 different species of annual herbs, some of which are edible (Peter & Gandhi, 2017). Some species of *Amaranthus,* such as *A. cruentus* and *A. hypochondriacus,* are consumed as grains and are highly nutritious (Alegbejo, 2013). Natural crude extracts of *Amaranthus* spp. have been used in traditional medicine to treat various ailments including urinary tract infections, gynecological disorders, diarrhea, pain, respiratory disorders, diabetes and also as a diuretic. Interest in *Amaranthus* (which means "immortal" in Greek) as a nutraceutical and natural protective agent against chronic diseases continues unabated. Many phytochemical studies undertaken to analyse various *Amaranthus* species have shown that they contain active constituents such as alkaloids, flavonoids, glycosides, phenolic acids, steroids, saponins, vitamins, terpenoids and carotenoids (Peter & Gandhi, 2017).

Amaranthus lividus L. (locally known as "dari mancari" in Turkey) is a vegetable whose stems and leaves are used for human consumption (Ozsoy, Yilmaz, Kurt, Can, & Yanardag, 2009). Limited studies conducted using different methods have thus far shown that extracts of *A. lividus* possess antioxidant activity (Al-Mamun et al., 2016; Amornrit & Santiyanont, 2016; Gunathilake & Ranaweera, 2016; Yilmaz-Ozden et al., 2016; Sarker, Oba, & Daramy, 2020). According to our literature search, no study has thus far been conducted on the enzyme inhibitory activity of *A. lividus*. The aim of this study is to search for new/natural α-amylase, α-glucosidase, AChE, elastase, lipase, neuraminidase and tyrosinase inhibitors by measuring *in vitro* enzyme inhibitory potential of *A. lividus*.

MATERIAL AND METHODS

Chemicals

α-Amylase, quercetin, kojic acid were purchased from Fluka Chemical Co. (Buchs, Switzerland). α-Glucosidase, AChE, acetylthiocholine iodide (ATChI), galantamine hydrobromide, 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB), neuraminidase, N-succinyl-Ala-Ala-Ala-p-anilide (STANA), tyrosinase, ursolic acid, p-nitrophenyl α-D-glucopyranoside (PNPG), 4-methylumbelliferyl-α-DN-acetylneuraminic acid sodium salt hydrate, rutin, lipase, 4-nitrophenyl caprate, orlistat were purchased from Sigma-Aldrich (St. Louis, MO, USA). Elastase was purchased from Calbiochem. Starch were purchased from Merck & Co. (Darmstdat, Germany). All other reagents were of analytical grade.

Plant material

Amaranthus lividus L. were collected from Bartın, Turkey. Voucher specimen was deposited at the Herbarium of the Faculty of Pharmacy, Istanbul University, Istanbul, Turkey (ISTE 83401).

Preparation of the extract

Plant materials were washed and dried at room temperature. The dried stems, leaves and flowers of *A. lividus* were manually ground to a fine powder. A water extract was prepared by heating the powdered plant material (10 g) with 100 mL distilled water for 30 minutes, stirring constantly. The extract was filtered and evaporated to dryness under reduced pressure at 40°C. The extract was stored at -20°C and dissolved in distilled water before use.

α-Amylase inhibitory activity

The inhibitory activity of *A. lividus* on α-amylase (from hog pancreas) was determined according to the modified method of Al-Dabbas, Kitahara, Suganuma, Hashimoto, & Tadera, (2006). The extract (30 µL) was mixed with 400 µg/mL soluble starch (100 µL) in phosphate buffer (0.25 M, pH 7.0). After 5 min of incubation at 37°C, 50 µg/mL α-amylase solution (10 µL) was added to the mixture, followed by the addition of 10 µL phosphate buffer (0.25 M, pH 7.0). After further incubation of the mixture for 7.5 min, 0.01 N iodine solution (100 µL) and then distilled water (500 µL) were added, followed by measurement of absorbance at 660 nm. Rutin was used as the standard compound. The percentage inhibition of enzyme activity was calculated according to the following formula:

$$\alpha - Amylase\ inhibitory\ activity\ (\%) = \left(\frac{A0 - A1}{A0}\right) \times 100$$

A_0 is the enzyme activity without an inhibitor.

A_1 is the enzyme activity in presence of an inhibitor.

α-Glucosidase inhibitory activity

The inhibitory activity of *A. lividus* on α-glucosidase (from *Saccharomyces cerevisieae*) was determined according to the modified method of Matsui, Yoshimoto, Osajima, Oki, & Osajima, (1996), in which the enzyme solution was adjusted to 32 mU/assay volume of α-glucosidase in a phosphate buffer (50 mM, pH 7.0) containing 100 mM NaCl. For each assay, the extract (10 µL) and the enzyme solution (40 µL) were pre-incubated for 5 min at 37°C. The reaction was started by adding 0.7 mM PNPG (950 µL) in buffer, and the solution was then incubated for 5 min at 37°C. After the reaction was stopped by adding 0.5 M Tris (1.0 mL), the absorbance of p-nitrophenyl released from PNPG was measured spectrophotometrically at 400 nm. Acarbose was used as the standard compound. The percent of enzyme activity inhibition was calculated according to the formula given below:

$$\alpha - Glucosidase\ inhibitory\ activity\ (\%) = \left(\frac{A0 - A1}{A0}\right) \times 100$$

A_0 is the enzyme activity without an inhibitor.

A_1 is the enzyme activity in presence of an inhibitor.

Acetylcholinesterase (AChE) inhibitory activity

The inhibitory activity of *A. lividus* on AChE was determined by the modified method of Ellman, Courtney, Andres Jr, & Featherstone, (1961). The extract (20 µL) and Ellman solution (220 µL; 318 mM DTNB, and 955 mM ATChI in phosphate buffer; pH 7.5) were mixed. Then, 0.5 U/mL AChE solution (10 µL) was added and the absorbance change was monitored at 412 nm for 10 min. A sample without inhibitor was treated the same and used as a negative control. Galantamine was used as a standard substance. The percentage inhibition of enzyme activity was calculated according to the following formula:

$$AChE\ inhibitory\ activity\ (\%) = \left(\frac{\text{Rate of control reaction} - \text{Rate of sample reaction}}{\text{Rate of control reaction}}\right) \times 100$$

Elastase inhibitory activity

Elastase inhibitory activity was determined using STANA as substrate and by measuring the realase of p-nitroanilide at 410 nm (James, Timothy, & Gordon, 1996). The extract (50 µL) was preincubated with 0.16 U/mL elastase solution (50 µL) and 900 µL of Tris-HCl buffer (0.2 M, pH 7.8) at 37°C for 15 min before addition of the substrate solution. Then 5 mM STANA solution (50 µL) was added and the mixture was incubated at 37°C for 30 min. The release of p-nitroaniline was measured at 410 nm. Ursolic acid was used as a standard. The percentage inhibition of elastase was calculated using the following formula:

$$Elastase\ inhibitory\ activity\ (\%) = \left(\frac{A0 - A1}{A0}\right) \times 100$$

A_0 is the enzyme activity without an inhibitor.

A_1 is the enzyme activity in presence of an inhibitor.

Lipase inhibitory activity

Lipase inhibitory activity was determined according to the method of Conforti et al. (2012). Pig pancreatic-type II lipase was prepared by dissolving 10 mg/mL of the enzyme in 0.1 M Tris-HCl buffer (pH 8.5), followed by centrifugation at 7000 g, for 10 min, and the collected supernatants were stored at -20°C until use (Lehner & Verger, 1997). Lipase activity was determined by measuring the hydrolysis of 4-nitrophenyl caprate to 4-nitrophenol. The reaction solution containing the extract (5 µL), 10 mg/mL lipase (10 µL), and 200 µL of Tris-HCl buffer (0.1 M, pH 8.5) was incubated at 37°C for 25 min, after which 5 mM 4-nitrophenyl caprate (5 µL) was added. The absorbance was read at 405 nm after incubation at 37°C for 15 min using a microplate reader. Orlistat was used as the standard inhibitor of lipase in this study. Percent lipase inhibition was calculated as follows:

$$Lipase\ inhibitory\ activity\ (\%) = \left(\frac{A0 - A1}{A0}\right) \times 100$$

A_0 is the enzyme activity without an inhibitor.

A_1 is the enzyme activity in presence of an inhibitor.

Neuraminidase inhibitory activity

The inhibitory activity of *A. lividus* on neuraminidase (from *Clostridium perfringens*) was determined according to the modified method of Myers et al. (1980). The reaction was carried out with 2.5 x 10^{-3} U/mL the enzyme solution (10 µL), 360 µL of acetate buffer (pH 5.0), the extract (10 µL) and 4-methylumbelliferyl-α-DN-acetylneuraminic acid sodium salt hydrate (20 µL). The mixture were incubated for 10 min at 37°C. After the reaction was stopped by adding 3.5 mL of glycine-NaOH buffer (pH 10.4), the fluorescence of the reactions was measured spectrofluorometrically. The emission wavelength was 440 nm, while the excitation wavelength was 360 nm. Quercetin was used as the standard compound. The percentage of inhibition was determined by the following equation:

$$Neuraminidase\ inhibitory\ activity\ (\%) = \left(\frac{\text{Rate of control reaction} - \text{Rate of sample reaction}}{\text{Rate of control reaction}}\right) \times 100$$

Tyrosinase inhibitory activity

Tyrosinase inhibitory activity was determined spectrophotometrically (Vanni et al., 1990). Assay reaction mixtures were prepared by adding 40 U mushroom tyrosinase (20 µL), the extract (20 µL), 1.5 mM L-tyrosine (40 µL) and 220 µL of sodium phosphate buffer (0.1 M, pH 6.5). The resulting mixture was incubated for 10 min at 37°C and absorbance value was measured at 475 nm. Kojic acid was used as the standard compound. The percent inhibition of tyrosinase was determined according to the following equation:

$$Tyrosinase\ inhibitory\ activity\ (\%) = \left(\frac{A0 - A1}{A0}\right) \times 100$$

A_0 is the enzyme activity without an inhibitor.

A_1 is the enzyme activity in presence of an inhibitor.

RESULTS AND DISCUSSION

α-Amylase inhibitory activity

One of the approaches to treating type 2 diabetes is to reduce the rate of blood glucose absorption from the small intestine by slowing the digestion of starch. α-Amylases are hydrolytic enzymes that are secreted in the mouth and small intestine. The function of α-amylases is to catalyze the hydrolysis of starch and lead to the production of maltose, maltotriose, maltotetraose, maltodextrins and glucose (Papoutsis et al., 2021). Inhibition of α-amylase activity helps to maintain circulating glucose at normal levels. In this study, α-amylase inhibitory activity of *A. lividus* was evaluated and rutin was used as a standard inhibitor. The results were expressed as IC_{50} value (the extract/standard concentration that inhibits enzyme activity by 50%) (Table 1). Low IC_{50} values indicate higher inhibitory potential of the tested samples. The water extract showed high α-amylase inhibitory activity compared to rutin. Rutin showed about 55% enzyme inhibition at 2.5 mg/mL, while the extract produced the same inhibition at a concentration of 1.5 mg/mL. According to our literature search, there has as yet been no study on the α-amylase inhibitory activity of *A. lividus*. However, there are a limited number of studies on the α-amylase inhibitory activity of other *Amaranthus* species. One such study reported that the methanol extract of *A. spinosus* significantly inhibited α-amylase activity (IC_{50} 46.02 µg/mL) (Kumar et al., 2011). Kumar et al. (2012) showed that methanol extract of *A. viridis* exhibited α-amylase inhibitory activity (IC_{50} 10.19 µg/mL). Another study reported that the water extracts of *A. gangeticus* and *A. inamoenus*, showed high α-amylase inhibitory activity at a concentration of 1 mg (Yang, Mong, Wu, Wang, & Yin, 2020). These findings are in agreement with the present data that *Amaranthus* extracts show good α-amylase inhibitory activity.

α-Glucosidase inhibitory activity

Oral antidiabetic agents, which act as competitive/reversible inhibitor on α-glucosidases, slow carbohydrate digestion and resulting in a decrease in postprandial blood glucose levels (Elbashir et al., 2018). In the present study, the water extract showed quite a good α-glucosidase inhibitory activity as compared to acarbose (Table 1). The water extract caused 73.28±0.14% enzyme inhibition at 0.01 mg/mL concentration, while acarbose caused only 21.28±4.25% at the same concentration. According to our literature search, there are two studies on the α-glucosidase inhibitory activity of *Amaranthus* species. A study by Mondal, Guria, & Maity, (2015) reported that methanol extract of *A. spinosus* showed significant α-glucosidase inhibitory activity (IC_{50} 8.49 µM/mL) and that acarbose provides inhibition at IC_{50} values of 15.25 µM/mL. Yang, Mong, Wu, Wang, & Yin (2020) reported that the water extracts of *A. gangeticus* and *A. inamoenus* showed high α-glucosidase inhibi-

Table 1. α-Amylase, α-glucosidase, acetylcholinesterase (AChE) and elastase inhibitory activities of water extract of *A. lividus*.

	IC_{50} Values (mg/mL)*			
	α-Amylase	**α-Glucosidase**	**AChE**	**Elastase**
A. lividus	0.0103±0.00057	0.0068±0.0001	247.66±54.90	0.69±0.12
Standards	**Rutin**	**Acarbose**	**Galantamine**	**Ursolic Acid**
	0.96±0.15	0.069±0.005	0.0092±0.0002	0.0065±0.0001

*Values represent the means of three replicates±standard deviation. IC_{50} values indicate the extract/standard concentration that inhibits enzyme activity by 50%. IC_{50} values were calculated from dose-response curves (by plotting the percentage of inhibition against concentration) using Microsoft Excel software.

tory activity (80-50%) at a concentration of 1 mg. Considering the results of other studies, it can be said that the extracts of *Amaranthus* species are good α-glucosidase inhibitors.

Acetylcholinesterase (AChE) inhibitory activity

The use of AChE inhibitors is an effective therapeutic approach to alleviate the symptoms of Alzheimer's disease (Silva et al., 2019). In this study, the water extract showed weak AChE inhibitory activity compared to galantamine (Table 1). According to our literature search, there has been no study as yet on AChE inhibitory activity of *A. lividus*. However, there are a few studies on AChE inhibitory activity of other *Amaranthus* species. As a result of the study conducted by Lee, Song, & Lee (2010), it was found that the extracts of water, 80% methanol and 70% ethanol (1 mg/mL) obtained from *A. mangostanus* did not cause AChE inhibition. Another study reported that the water extracts of *A. gangeticus* and *A. inamoenus*, showed moderate anti-AChE activity (50-60%) at a concentration of 1 mg (Yang, Mong, Wu, Wang, & Yin, 2020). In a study on *A. tricolor*, traditionally used as a memory enhancer in Anatolia (Orhan & Aslan, 2009), it was reported that the extract of the aerial parts (0.5 mg/mL) of *A. tricolor* showed 3% AChE inhibitory activity (Lobbens, Vissing, Jorgensen, van de Weert, & Jäger, 2017). However, another study by Hupparage et al. (2020) reported that the ethanolic extract of *A. tricolor* (leaves) showed good AChE inhibition (IC_{50} value 193.9 µg/mL). Nuria, Suganda, Sukandar, & Insanu (2020) reported that ethanol extract of *A. cruentus* (leaves) at 1 mg/mL exhibited AChE inhibitory activity of 22.6±0.7%. A study by Ishtiaq et al. (2017) reported that methanol extract of *A. graecizans* subsp. *silvestris* showed mild AChE inhibition (24.29±0.57%). In agreement with our findings, other *Amaranthus* species have also been reported to no significant AChE inhibitory activity.

Elastase inhibitory activity

The increase in elastase expression due to excessive UV irradiation and oxidative damage can lead to decreased skin elasticity and induce wrinkling and sagging. Plants contain many natural substances that can be used as anti-aging agents, and also to treat dermatological conditions. It has been suggested that skin aging and anti-wrinkle effects are directly related to the breakdown of elastin, thus many studies have investigated the elastase inhibitory activity of plants (Liyanaarachchi, Samarasekera, Mahanama, & Hemalal, 2018). In this study, the water extract of *A. lividus* showed good elastase inhibitory activity, although not as good as ursolic acid (Table 1). While the water extract at 2 mg/mL showed inhibition of 71.93±0.25%, ursolic acid at 0.001 mg/mL showed inhibition of 72.78±1.02%. According to our literature search, there has not as yet any study on elastase inhibitory activity of *Amaranthus* species.

Lipase inhibitory activity

Lipase inhibitors work by reducing the absorption of dietary fats and are thus effective therapeutics for obesity and hyperlipidemia (Elbashir et al., 2018). In the present study, the water extract showed good lipase inhibitory activity, although not as good as orlistat (Table 2). According to our literature search, there has as yet been no study on lipase inhibitory activity of *A. lividus*. However, there is a single study on the lipase inhibitory activity of other *Amaranthus* species. Yang, Mong, Wu, Wang, & Yin (2020) reported that the water extracts of *A. gangeticus* and *A. inamoenus*, showed good anti-lipase activity (70-50%) at a concentration of 1 mg. The results of the study are in agreement with our findings, namely that the water extract of *A. lividus* at 1 mg/mL caused 60.38±0.91% lipase inhibition.

Neuraminidase inhibitory activity

Neuraminidase inhibitors such as zanamivir and oseltamivir have been used as antiviral drugs to treat influenza. However, due to the development of resistance to these agents, new, natural antiviral product compounds based on neuraminidase inhibitory activity are needed for the treatment of influenza (Kwak et al., 2018). In the present study, the water extract of *A. lividus* showed quite low neuraminidase inhibitory activity compared to quercetin (Table 2). According to our literature search, there has not any study as yet on the neuraminidase inhibitory activity of *Amaranthus* species.

Tyrosinase inhibitory activity

Tyrosinase inhibitors are significant in cosmetics and pharmaceuticals as whitening agents and for the therapy of pigmentary disorders (Liyanaarachchi, Samarasekera, Mahanama, & Hemalal, 2018). In this study, the water extract of *A. lividus* showed good tyrosinase inhibitory activity, although not as good as kojic acid (Table 2). According to our literature search, there has as yet been no study on tyrosinase inhibitory activity of *A. lividus*. Moreover, there are only three studies on tyrosinase inhibitory activity of other *Amaranthus* species. In one study, an 80% methanol extract of *A. magnostanus* (aerial parts) showed 8% tyrosinase inhibition at a concentration of 100 µg/mL (Shin et al., 1997). Li & Wang (2014)'s study reported that methanol extract of *A.*

Table 2. Lipase, neuraminidase and tyrosinase inhibitory activities of water extract of *A. lividus*.

	IC_{50} Values (mg/mL)*		
	Lipase	**Neuraminidase**	**Tyrosinase**
A. lividus	0.56±0.06	1.48±0.23	2.27±0.08
Standards	**Orlistat**	**Quercetin**	**Kojic Acid**
	0.0009±0.00005	$45.67x10^{-6}$±$3.52x10^{-6}$	0.68±0.07

*Values represent the means of three replicates ± standard deviation. IC_{50} values indicate the extract/standard concentration that inhibits enzyme activity by 50%. IC_{50} values were calculated from dose-response curves (by plotting the percentage of inhibition against concentration) using Microsoft Excel software.

viridis (whole plant) has no α-tyrosinase inhibitory activity. In another study, the methanol extract of *A. viridis* showed higher anti-tyrosinase activity (IC_{50} 36.31 µg/mL) as compared to the extracts followed by chloroform extract (IC_{50} 57.12 µg/mL), water extract (IC_{50} 62.32 µg/mL) and hexane extract (IC_{50} 87.14 µg/mL), (Kumari, Elancheran, & Devi, 2018). In a study on tyrosinase inhibition of *A. albus* (Badem et al., 2018), the methanol extract was used at concentrations of 25, 50, 100 and 500 µg/mL, and the extract showed so low an inhibitory effect that the IC_{50} value could not be calculated.

In addition, there are a limited number of studies on tyrosinase inhibitory activity of *Amaranth* seeds. Seo et al. (2016) reported the inhibitory effect of *Amaranthus* spp. seed extract on tyrosinase activity in melan-a cells. The results showed that the water extract of a species crossed between *A. hybridus* and *A. hypocondriacus* showed significant inhibition of tyrosinase activity similar to that of kojic acid. A study by Yi, Kang, & Bu (2017) reported the tyrosinase inhibitory activities of 70% ethanol extract and subfractions of *Amaranth* seeds. The ethyl acetate fraction of the seeds showed stronger tyrosinase inhibitory activity than that of the positive control cynandione A at 50, 100 and 200 µg/mL. At a concentration of 50 µg/mL, cynandione A produced about 50% tyrosinase inhibition, while the ethyl acetate fraction produced about 65% inhibition. Finally, the study by Rocchetti et al. (2020) reported that *Amaranthus* microgreens have potential to inhibit tyrosinase activity.

CONCLUSIONS

Amaranthus spp. have been used in medicine from ancient times to the present for the treatment of various diseases, and the species also continues to be the subject of research. Although some activities of *A. lividus* have been reported in the literature, no study has been carried out on the inhibitory activity of α-amylase, α-glucosidase, AChE, elastase, lipase, neuraminidase and tyrosinase. In the present study, the inhibitory potential of *A. lividus* against some therapeutic target enzymes was investigated and brought to the literature for the first time. In conclusion, the water extract of *A. lividus* exhibited significant and promising α-glucosidase and α-amylase inhibitory activity compared to standard inhibitors. Also, the extract displayed good elastase, lipase and tyrosinase inhibitory activities. However, it exhibited low inhibitory activity on AChE and neuraminidase. In particular, due to antidiabetic activity of *A. lividus*, the plant and its active constituents can be used as a therapeutic enzyme inhibitor with future phytochemical constituent analysis.

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REFERENCES

- Al-Dabbas, M. M., Kitahara, K., Suganuma, T., Hashimoto, F., & Tadera, K. (2006). Antioxidant and α-amylase inhibitory compounds from aerial parts of *Varthemia iphionoides* Boiss. *Bioscience, Biotechnology, and Biochemistry*, *70*, 2178–2184.
- Alegbejo, J. O. (2013). Nutritional value and utilization of *Amaranthus* (*Amaranthus* spp.)-A review. *Bayero Journal of Pure and Applied Sciences*, *6*(1), 136–143.
- Al-Mamun, M. A., Husna, J., Khatun, M., Hasan, R., Kamruzzaman, M., Hoque, K., Reza, M. A., & Ferdousi, Z. (2016). Assessment of antioxidant, anticancer and antimicrobial activity of two vegetable species of *Amaranthus* in Bangladesh. *BMC Complementary and Alternative Medicine*, *16*, 157. Doi: 10.1186/s12906-016-1130-0.
- Amornrit, W., & Santiyanont, R. (2016). Neuroprotective effect of *Amaranthus lividus* and *Amaranthus tricolor* and their effects on gene expression of RAGE during oxidative stress in SH-SY5Y cells. *Genetics and Molecular Research*, *15*, 15027562.
- Badem, M., Korkmaz, N., Sener, S. O., Kanbolat, S., Ozgen, U., Sevgi, S., Aliyazicioglu, R., & Coskun, M. (2018). Biological screening of traditional medicinal plants from villages of Akkuş (Ordu) in Turkey on the effects of tyrosinase. *Journal of Pharmaceutical Research International*, *25*(6), 1–10.
- Chatatikun, M., Yamauchi, T., Yamasaki, K., Aiba, S., & Chiabchalard A. (2019). Anti melanogenic effect of *Croton roxburghii* and *Croton sublyratus* leaves in α-MSH stimulated B16F10 cells. *Journal of Traditional Complementary Medicinal*, *9*(1), 66–72.
- Chiocchio, I., Mandrone, M., Sanna, C., Maxia, A., Tacchini, M., & Poli, F. J. I. C. (2018). Screening of a hundred plant extracts as tyrosinase and elastase inhibitors, two enzymatic targets of cosmetic interest. *Industrial Crops and Products*, *122*, 498–505.
- Conforti, F., Perri, V., Menichini, F., Marrelli, M., Uzunov, D., Statti, G., & Menichini, F. (2012). Wild Mediterranean dietary plants as inhibitors of pancreatic lipase. *Phytotherapy Research*, *26*, 600–604.
- Elbashir, S. M. I., Devkota, H. P., Wada, M., Kishimoto, N., Moriuchi, M., Shuto, T., Misumi, S., Kai, H., & Watanabe, T. (2018). Free radical scavenging, α-glucosidase inhibitory and lipase inhibitory activities of eighteen Sudanese medicinal plants. *BMC Complementary and Alternative Medicine*, *18*(1), 1–12.
- Ellman, G. L., Courtney, K. D., Andres Jr, V., & Featherstone, R. M. (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemical Pharmacology*, *7*(2), 88–95.
- Gunathilake, K. P. P., & Ranaweera, K. (2016). Antioxidative properties of 34 green leafy vegetables. *Journal of Functional Foods*, *26*, 176–186.
- Hupparage, V. B., Rasal, V. P., Patil, V. S., Patil, P. P., Mulange, S. G., Malgi, A. P., Patil, S. A., & Karade, A. R. (2020). Ameliorative effect of *Amaranthus tricolor* L. leaves on scopolamine-induced cognitive dysfunction and oxidative stress in rats. *Journal of Applied Pharmaceutical Science*, *10*(10), 111–120.
- Ishtiaq, S., Ali, T., Ahmad, B., Anwar, F., Afridi, M. S. K., & Shaheen, H. (2017). Phytochemical and biological evaluations of methanolic extract of *Amaranthus graecizans* subsp. silvestris (Vill.) Brenan. *Journal of Pharmaceutical Research International*, *15*(3), 1–11.
- James, A. E., Timothy, D. W., & Gordon, L. (1996). Inhibition of human leukocyte and pancreatic elastase by homologues of bovine pancreatic trypsin inhibitors. *Biochemistry*, *35*, 9090–9096.

- Kumar, B. A., Lakshman, K., Jayaveea, K. N., Shekar, D. S., Khan, S., Thippeswamy, B. S., & Veerapur, V. P. (2012). Antidiabetic, antihyperlipidemic and antioxidant activities of methanolic extract of *Amaranthus viridis* Linn in alloxan induced diabetic rats. *Experimental and Toxicologic Pathology*, *64*(1-2), 75–79.
- Kumar, B. A., Lakshman, K., Nandeesh, R., Kumar, P. A., Manoj, B., Kumar, V., & Shekar, D. S. (2011). *In vitro* alpha-amylase inhibition and *in vivo* antioxidant potential of *Amaranthus spinosus* in alloxan-induced oxidative stress in diabetic rats. *Saudi Journal of Biological Sciences*, *18*(1), 1–5.
- Kumari, S., Elancheran, R., & Devi, R. (2018). Phytochemical screening, antioxidant, antityrosinase, and antigenotoxic potential of *Amaranthus viridis* extract. *Indian Journal of Pharmacology*, *50*(3), 130–138.
- Kwak, H. J., Park, S., Kim, N., Yoo, G., Park, J. H., Oh, Y., Nhiem, N. X., & Kim, S. H. (2018). Neuraminidase inhibitory activity by compounds isolated from aerial parts of *Rhinacanthus nasutus*. *Natural Product Research*, *32*(17), 2111–2115.
- Lee, E. N., Song, J. H., & Lee, J. S. (2010). Screening of a potent antidementia acetylcholinesterase inhibitor-containing fruits and optimal extraction conditions. *The Korean Journal of Food and Nutrition*, *23*(3), 318–323.
- Lee, N. & Hurt, A. C. (2018). Neuraminidase inhibitor resistance in influenza: A clinical perspective. *Current Opinion in Infectious Diseases*, *31*(6), 520–526.
- Lehner, R. & Verger, R. (1997). Purification and characterization of a porcine liver microsomal triacylglycerol hydrolase. *Biochemistry,* 36, 1861–1868.
- Li, C. & Wang, M. H. (2014). *In vitro* biological evaluation of 100 selected methanol extracts from the traditional medicinal plants of Asia. *Nutrition Research and Practice*, *8*(2), 151–157.
- Liu, T. T., Liu, X. T., Chen, Q. X., & Shi, Y. (2020). Lipase inhibitors for obesity: A review. *Biomedicine & Pharmacotherapy*, 128, 110314.
- Liyanaarachchi, G. D., Samarasekera, J. K. R. R., Mahanama, K. R. R., & Hemalal, K. D. P. (2018). Tyrosinase, elastase, hyaluronidase, inhibitory and antioxidant activity of Sri Lankan medicinal plants for novel cosmeceuticals. *Industrial Crops and Products*, *111*, 597–605.
- Lobbens, E. S., Vissing, K. J., Jorgensen, L., van de Weert, M., & Jäger, A. K. (2017). Screening of plants used in the European traditional medicine to treat memory disorders for acetylcholinesterase inhibitory activity and anti amyloidogenic activity. *Journal of Ethnopharmacology*, *200*, 66–73.
- Mahal, A., Duan, M., Zinad, D. S., Mohapatra, R. K., Obaidullah, A. J., Wei, X., Pradhan, M. K., Das, D., Kandi, V., Zinadjk, H. S., & Zhu, Q. (2021). Recent progress in chemical approaches for the development of novel neuraminidase inhibitors. *RSC Advances*, *11*(3), 1804–1840.
- Matsui, T., Yoshimoto, C., Osajima, K., Oki, T., & Osajima,Y. (1996). *In vitro* survey of alpha-glucosidase inhibitory food components. *Bioscience, Biotechnology, and Biochemistry*, *60*, 2019–2022.
- Mondal, A., Guria, T., & Maity, T. K. (2015). A new ester of fatty acid from a methanol extract of the whole plant of *Amaranthus spinosus* and its α-glucosidase inhibitory activity. *Pharmaceutical Biology*, *53*(4), 600–604.
- Myers R. W., Lee R. T., Lee Y. C., Thomas G. H., Reynolds L. W., & Uchida Y. (1980). The synthesis of 4-methylumbelliferyl α-ketoside of N-acetylneuraminic acid and its use in a fluorometric assay for neuraminidase. *Analytical Biochemistry*, *101*, 166–174.
- Nuria, M. C., Suganda, A. G., Sukandar, E. Y., & Insanu, M. (2020). Acetylcholinesterase: Inhibitory activity of some Indonesian vegetables and fraction of selected plants. *Journal of Applied Pharmaceutical Science*, *10*(1), 101–107.
- Orhan, I., & Aslan, M. (2009). Appraisal of scopolamine-induced antiamnesic effect in mice and *in vitro* antiacetylcholinesterase and antioxidant activities of some traditionally used Lamiaceae plants. *Journal of Ethnopharmacology*, *122*(2), 327–332.
- Ozsoy, N., Yilmaz, T., Kurt, O., Can, A., & Yanardag, R. (2009). *In vitro* antioxidant activity of *Amaranthus lividus* L. *Food Chemistry*, *116*(4), 867–872.
- Papoutsis, K., Zhang, J., Bowyer, M. C., Brunton, N., Gibney, E. R., & Lyng, J. (2021). Fruit, vegetables, and mushrooms for the preparation of extracts with α-amylase and α-glucosidase inhibition properties: A review. *Food Chemistry*, *338*, 128119.
- Peter, K. & Gandhi, P. (2017). Rediscovering the therapeutic potential of *Amaranthus* species: A review. *Egyptian Journal of Basic and Applied Sciences*, *4*(3), 196–205.
- Ramsay, R. R. & Tipton, K. F. (2017). Assessment of enzyme inhibition: A review with examples from the development of monoamine oxidase and cholinesterase inhibitory drugs. *Molecules*, *22*(7), 1192. Doi: 10.3390/molecules22071192.
- Rauf, A., & Jehan, N. (2017). Natural products as a potential enzyme inhibitors from medicinal plants. In Şentürk., M. (Eds.), *Enzyme Inhibitors and Activators* (pp. 165-177). Rijeka, Croatia, InTech.
- Rocchetti, G., Tomas, M., Zhang, L., Zengin, G., Lucini, L., & Capanoglu, E. (2020). Red beet (*Beta vulgaris*) and amaranth (*Amaranthus* sp.) microgreens: Effect of storage and *in vitro* gastrointestinal digestion on the untargeted metabolomic profile. *Food Chemistry*, *332*, 127415. Doi: 10.1016/j.foodchem.2020.127415
- Santos, T. C. D., Gomes, T. M., Pinto, B. A. S., Camara, A. L., & Paes, A. M. D. A. (2018). Naturally occurring acetylcholinesterase inhibitors and their potential use for Alzheimer's disease therapy. *Frontiers in Pharmacology*, *9*, 1192. Doi: 10.3389/fphar.2018.01192.
- Sarker, U. Oba, S., & Daramy, M. A. (2020). Nutrients, minerals, antioxidant pigments and phytochemicals, and antioxidant capacity of the leaves of stem Amaranth. *Scientific Reports*, *10*, 1–9.
- Seo, J. O., Do, M. H., Lee, J. H., Lee, T. H., Wahedi, H. M., Park, Y. U., & Kim, S. Y. (2016). Modulation of melanin synthesis by *Amaranthus* spp. L seed extract in melan-a cells. *Natural Product Sciences*, *22*(3), 168–174.
- Shin, N. H., Lee, K. S., Kang, S. H., Min, K. R., Lee, S. H., & Kim, Y. S. (1997). Inhibitory effects of herbal extracts on dopa oxidase activity of tyrosinase. *Natural Product Sciences*, *3*(2), 111–121.
- Silva, S. G., da Costa, R. A., de Oliveira, M. S., da Cruz, J. N., Figueiredo, P. L. B., Brasil, D. D. S. B., Nascimento, L. D., Neto, A. M. J. C., Junior, R. N. C., & Andrade, E. H. D. A. (2019). Chemical profile of *Lippia thymoides*, evaluation of the acetylcholinesterase inhibitory activity of its essential oil, and molecular docking and molecular dynamics simulations. *PLoS One*, *14*(3), e0213393.
- Şöhretoğlu, D., & Sari, S. (2020). Flavonoids as alpha-glucosidase inhibitors: Mechanistic approaches merged with enzyme kinetics and molecular modelling. *Phytochemistry Reviews*, *19*, 1081–1092.
- Vanni, A., Gastaldi, D., & Gıunata, G. (1990). Kinetic investigations on the double enzymatic activity of the tyrosinase mushroom. *Annales des Chimie et des Physique*, *80*, 35–60.
- Wang, Z., Cheng, L. P., Zhang, X. H., Pang, W., Li, L., & Zhao, J. L. (2017). Design, synthesis and biological evaluation of novel oseltamivir derivatives as potent neuraminidase inhibitors. *Bioorganic & Medicinal Chemistry Letters*, *27*(24), 5429–5435.
- Yang, Y.C., Mong, M.C., Wu, W.T., Wang, Z.H., & Yin, M.C. (2020). Phytochemical profiles and anti-diabetic benefits of two edible *Amaranthus* species. *CyTA-Journal of Food*, *18*(1), 94-101.
- Yi, M. R., Kang, C. H., & Bu, H. J. (2017). Anti-inflammatory and tyrosinase ınhibition effects of Amaranth (*Amaranthus* spp L.) seed extract. *Korean Journal of Plant Resources*, *30*(2), 144–151.
- Yilmaz-Ozden, T., Can, A., Karatug, A., Pala-Kara, Z., Okyar, A., & Bolkent, S. (2016). Carbon tetrachloride-induced kidney damage and protective effect of *Amaranthus lividus* L. in rats. *Toxicology and Industrial Health, 32*, 1143–1152.
- Zolghadri, S., Bahrami, A., Hassan Khan, M. T., Munoz-Munoz, J., Garcia-Molina, F., Garcia-Canovas, F., & Saboury, A. A. (2019). A comprehensive review on tyrosinase inhibitors. *Journal of Enzyme Inhibition and Medicinal Chemistry*, *34*(1), 279–309.