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A study on *in vitro* enzyme inhibitory properties of *Asphodeline anatolica*: New sources of natural inhibitors for public health problems

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ABSTRACT

Asphodeline species are traditionally used as food and medicines. The different extracts (acetone, methanol and water) from different parts (stem, root, seed and leaf) of *Asphodeline anatolica* were screened for inhibitory potentials on cholinesterase, tyrosinase, α-amylase and α-glucosidase. Enzyme inhibitory effects were investigated by using microplate reader. All studied extracts exhibited remarkable inhibitory effects on the tested enzymes. Generally, acetone and methanol extracts have higher potentials than water extracts. Also, the enzyme inhibitory activities of the extracts varied significantly according to the plant parts as well as the solvent used. R-Met (7.42 mgGALAEs/g extract) and St-Ac (10.74 mgGALAEs/g extract) had the highest acetylcholinesterase and butrylcholinesterase inhibitory activity, respectively. R-Met (22.48 mgKAEs/g extract) exhibited the strongest tyrosinase inhibitory effects, while Se-Ac had the most potent activity on both α-amylase (2.53 mmolACAEs/g extract) and α-glucosidase (6.70 mmolACAEs/g extract). The results suggested that the *A. anatolica* extract may be useful for food and medicinal applications.

1. Introduction

For many centuries, plants or plant products are vital sources for drug and pharmaceutical industries. About 20% of the drugs used worldwide come from plants. The main reasons for this can be summarized as; they are easily and cheaply obtained (1) and they have stronger and safe activities than synthetics (2) (Rates, 2001). Currently, several natural products provide important drugs for many chronic and degenerative global health problems including cancer, Alzheimer diseases and diabetes mellitus (Orhan and Şener, 2006). In this context, there has been growing interest in the exploration of new natural products derived from plants.

The genus *Asphodeline* is classified in the family Xanthorrhoeaceae and represented by 14 species in the world. In Turkey, the genus contains 20 taxa, 12 taxa of which are endemic to Turkey (Mathews and Tuzlaci, 1984; Tuzlaci, 1987). In this direction, Turkey is considered as the primary gene center of this genus. The members of *Asphodeline* (*Asphodeline damascena, Asphodeline cilicica* and *Asphodeline globifera*) are traditionally used for the treatment of various disorders such as haemorrhoids and earaches. Moreover, some *Asphodeline* species (*Asphodeline lutea* and *Asphodeline taurica*) are commonly used as foodstuffs (Tuzlaci, 1985). Our previous studies showed that *Asphodeline* species are good nutritional quality and significant biological effects including antioxidant, antiproliferative and enzyme inhibitory effects. These studies are summarized in Table 1. However, to the best of our knowledge, there is no report about enzyme inhibitory effects of *Asphodeline anatolica* parts. Thus, the present work was aimed to assess the enzyme inhibitory effects (anti-cholinesterase, anti-tyrosinase, anti-amylase and anti-glucosidase) of different extracts from different parts of *A. anatolia*, which is endemic to Turkey. The obtained results may be interest of prepare new natural products in food and pharmaceutical area.

2. Materials and methods

2.1. Plant material and preparation of extracts

The herbal parts of *A. anatolica* E. Tuzlaci was collected from Sarkikaraagac-Yenisarbademli road, 38°03′07″ N, 31°17′51″E, 1144 m, Isparta-Turkey when the end of flowering season (July 2012). The voucher specimen was deposited at the KNYA Herbarium of Department of Biology, Selcuk University, Konya-Turkey (Voucher No: GZ 1001). The plant materials (stem, root, seed and leaf) were dried at room temperature. The dried parts were ground to a fine powder using a laboratory mill. Each of the powdered parts (10 g) were separately extracted with acetone and methanol

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Table 1
Some studies on several Asphodeline species.

Asphodeline species	Parameters	Results	References
A. lutea	Antioxidant properties	The chloroform and ethyl acetate extracts manifested strong antioxidant abilities	Lazarova et al. (2009)
A. lutea	Anthraquinones, naphthalene, and naphthoquinone components	New anthraquinones	Todorova et al. (2010)
Eleven *Asphodeline* species	Nutritionally quality of leaves protein	The *Asphodeline* leaves have good nutritional quality	Zengin et al. (2012)
A. anatolica	Antioxidant properties	Antioxidant properties showed differences in the used solvents and plant parts	Zengin and Aktumsek (2014)
A. lutea	Antioxidant properties and phenolic profiles	The extracts exhibited significant antioxidant properties. Caffeic acid was the major phenolic compounds	Lazarova et al. (2014)
A. lutea	HPLC-UV profiles, enzyme inhibitory and anti-proliferative properties	The extracts were potent enzyme inhibitor and antiproliferative agents	Lazarova et al. (2015)
Eight *Asphodeline* root extracts	Anthraquinones profiles, antioxidant and enzyme inhibitory properties	The extracts had important anthraquinones and exhibited remarkable antioxidant and enzyme inhibitory activities	Zengin et al. (2015)

Table 2
Enzyme inhibitory activities of different solvent extracts obtained from different parts of *A. anatolica*.*

Plant Parts	Solvents/ Abbreviations	Acetylcholinesterase (mg GALAEs/g extract)[a]	Butrylcholinesterase (mg GALAEs/g extract)[a]	Tyrosinase (mg KAEs/g extract)[b]	α-Amylase (mmol ACAEs/g extract)[c]	α-Glucosidase (mmol ACAEs/g extract)[c]
Stem	Acetone (St-Ac)	7.34 ± 0.01[a]	10.74 ± 0.06[a]	13.02 ± 0.43[a]	2.26 ± 0.01[a]	4.96 ± 0.12[b]
	Methanol (St-Met)	7.32 ± 0.01[a]	9.66 ± 0.02[a]	12.19 ± 1.82[a]	1.25 ± 0.03[b]	6.09 ± 0.02[a]
	Water (St-Wat)	3.48 ± 0.02[b]	4.08 ± 0.58[b]	14.76 ± 0.11[a]	0.22 ± 0.01[c]	0.53 ± 0.01[c]
Root	Acetone (R-Ac)	7.41 ± 0.01[a]	10.60 ± 0.01[a]	6.28 ± 0.32[c]	2.42 ± 0.01[a]	6.51 ± 0.06[a]
	Methanol (R-Met)	7.42 ± 0.01[a]	10.10 ± 0.02[a]	22.48 ± 1.39[a]	1.29 ± 0.05[b]	6.65 ± 0.14[a]
	Water (R-Wat)	3.58 ± 0.03[b]	3.93 ± 0.51[b]	17.03 ± 0.96[b]	0.28 ± 0.01[c]	0.62 ± 0.25[b]
Seed	Acetone (Se-Ac)	6.94 ± 0.01[b]	9.01 ± 0.20[a]	16.73 ± 0.54[b]	2.53 ± 0.02[a]	6.70 ± 0.01[a]
	Methanol (Se-Met)	7.34 ± 0.01[a]	9.10 ± 0.03[a]	16.43 ± 0.54[b]	1.05 ± 0.02[b]	5.57 ± 0.02[b]
	Water (Se-Wat)	3.33 ± 0.04[c]	2.97 ± 0.15[b]	20.89 ± 0.21[a]	0.29 ± 0.04[c]	3.19 ± 0.11[c]
Leaf	Acetone (L-Ac)	6.85 ± 0.01[b]	8.79 ± 0.18[a]	12.94 ± 2.03a	1.57 ± 0.01a	5.89 ± 0.13[a]
	Methanol (L-Met)	6.96 ± 0.01[a]	8.79 ± 0.20[a]	21.35 ± 3.43a	1.46 ± 0.02b	5.02 ± 0.24[b]
	Water (L-Wat)	2.85 ± 0.04[c]	1.65 ± 0.16[b]	16.80 ± 0.86a	0.41 ± 0.01c	0.27 ± 0.01[c]

* Data marked with different letter within the same column for each plant part indicate significant difference statistically ($p < 0.05$).
[a] GALAEs, galanthamine equivalents.
[b] KAEs, kojic acid equivalents.
[c] ACAEs, acarbose equivalents.

in a Soxhlet apparatus for 6–8 h. The extracts were concentrated under vacuum at 40 °C using a rotary evaporator. To obtain water extracts, the powdered samples were boiled with 250 mL of distilled water for 30 min. The aqueous extracts were filtered and lyophilized (−80 °C, 48 h). Extracts were stored at +4 °C in dark until use.

2.2. Enzyme inhibitory activity

2.2.1. Cholinesterase inhibition

Cholinesterase (ChE) inhibitory activity was measured using Ellman's method, as previously reported (Aktumsek et al., 2013) with slight modification. Sample solution (50 μL) was mixed with DTNB (125 μL) and AChE (or BChE) solution (25 μL) in Tris–HCl buffer (pH 8.0) in a 96-well microplate and incubated for 15 min at 25° C. The reaction was then initiated with the addition of acetylthiocholine iodide (ATCI) or butyrylthiocholine chloride (BTCl) (25 μL). Similarly, a blank was prepared by adding sample solution to all reaction reagents without enzyme (AChE or BuChE) solution. The sample and blank absorbances were read at 405 nm after a 10 min incubation at 25° C. The absorbance of the blank was subtracted from that of the sample and the cholinesterase inhibitory activity was expressed as equivalents of galanthamine (mgGALAEs/g extract). (Absorbance (A) = 0.0607 (μg galanthamine) +0.4746, (R^2 = 0.9404) for AChE; A = 1.5806 (μg galanthamine) +0.2839, (R^2 = 0.9993) for BChE).

2.2.2. Tyrosinase inhibition

Tyrosinase inhibitory activity was measured using the modified dopachrome method with L-DOPA as substrate, as previously reported (Zengin et al., 2014) with slight modification. Sample solution (25 μL) was mixed with tyrosinase solution (40 μL) and phosphate buffer (100 μL, pH 6.8) in a 96-well microplate and incubated for 15 min at 25 °C. The reaction was then initiated with the addition of L-DOPA (40 μL). Similarly, a blank was prepared by adding sample solution to all reaction reagents without enzyme (tyrosinase) solution. The sample and blank absorbances were read at 492 nm after a 10 min incubation at 25 °C. The absorbance of the blank was subtracted from that of the sample and the tyrosinase inhibitory activity was expressed as equivalents of kojic acid (mgKAEs/g extract). (A = 0.0775 (μg kojic acid) +0.0163, R^2 = 0.9974)

2.2.3. α-Amylase inhibition

α-Amylase inhibitory activity was performed using Caraway-Somogyi iodine/potassium iodide (IKI) method (Lazarova et al., 2015) with some modifications. Sample solution (25 μL) was mixed with α-amylase solution (50 μL) in phosphate buffer (pH 6.9 with 6 mM sodium chloride) in a 96-well microplate and incubated for 10 min at 37 °C. After pre-incubation, the reaction was initiated with the addition of starch solution (50 μL, 0.05%). Similarly, a blank was prepared by adding sample solution to all reaction reagents without enzyme (α-amylase) solution. The reaction mixture was