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Evaluation of Anticholinergic, Antidiabetic and Antioxidant Activity of *Astragalus dumanii*, an Endemic Plant

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ABSTRACT

The research was conducted to separately evaluate and detect the possible *in vitro* antioxidant, antimicrobial activity of ethanol extracts prepared from aerial parts and roots of *Astragalus dumanii* and anti-cholinesterase and α-glucosidase inhibitory activity from only aerial parts of its The antioxidant capacity was tested by scavenging of DPPH and ABTS free radicals. Compared with the standard antioxidant compound gallic acid; Root and aerial part extract showed lower DPPH radical scavenging activity, however aerial part extract demonstrated higher ABTS radical scavenging activity. The phenolic contents were detected as 5.31 ± 0.03 and 13.23 ± 0.05 mg gallic acid equivalent g^{-1} extract, flavonoid contents were found as 8.26 ± 0.004 and 7.93 ± 0.005 mg Qercetin equivalent g^{-1} extract. In addition, the effects of the extracts obtained from aerial parts of the plant on acetylcholinesterase, butyrylcholinesterase and a-glycosidase enzymes were investigated in vitro and IC_{50} values were obtained as 1.47, 0.83 and 0.48 µg mL^{-1}, respectively. When these values were compared with standard substances, it was seen that Astragalus *dumanii* could be a good enzyme inhibitory agent. Antimicrobial activity of the plant extracts were determined using the microdilution method and the extracts was not observed to have any antimicrobial activities..

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Sivas da Yetişen Endemik Bir Bitki Olan *Astragalus Dumanii*'nin Antikolinerjik, Antidiyabetik ve Antioksidan Aktivitesinin Değerlendirilmesi

ÖZET

Araştırma, *Astragalus dumanii* bitkisinin topraküstü ve köklerinden hazırlanan etanol ekstrelerinin olası *in vitro* antioksidan, antimikrobiyal ve yalnızca topraküstü etanol ekstrelerinin anti-kolinesteraz ve α-glikozidaz inhibitör aktivitesini ayrı ayrı değerlendirmek ve tespit etmek için yapılmıştır. Antioksidan kapasitesi, DPPH ve ABTS serbest radikalleri temizleme metoduyla test edildi. Standart antioksidan bileşik gallik asit ile karşılaştırıldığında; Kök ve toprak üstü kısım ekstreleri düşük DPPH radikal süpürücü aktivite gösterirken, toprak üstü kısmı daha yüksek ABTS radikal süpürücü aktivite göstermiştir. Fenolik içerikleri 5,31 ± 0,03 ve 13,23 ± 0,05 mg gallik asite eşdeğer g^{-1} ekstre, flavonoit içerikleri ise 8,26 ± 0,004 ve 7,93 ± 0,005 mg kersetine eşdeğer g^{-1} özüt olarak bulunmuştur. Ayrıca bitkinin toprak üstü kısımlarından elde edilen ekstrelerin asetilkolinesteraz, butirilkolinesteraz ve a-glikosidaz enzimlerine etkisi in vitro araştırıldı ve sırasıyla IC_{50} değerleri: 1.47, 0.83 ve 0.48 µg mL^{-1} elde

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Anahtar Kelimeler
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Antidiyabetik
Asetilkolinesteraz
Butirilkolinesteraz

edildi. Bu değerler stadart maddelerle karşılaştırıldığında da *Astragalus dumanii*'nin iyi bir enzim inhibe edici ajan olabileceği görülmüştür. Bitki ekstrelerinin antimikrobiyal aktiviteleri mikrodilüsyon yöntemi kullanılarak belirlendi ve ekstrelerde herhangi bir antimikrobiyal aktivite gözlemlenmedi.

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INTRODUCTION

Plants have been used from ancient times for maintaining good health and to cure various ailments. The genus *Astragalus* (Leguminosae) is represented by 2500 taxa and widely distributed in the world. It is also among the largest genus in Turkey, known as 'geven' in Turkish language and represented by 439 species in 61 sections (Chamberlain and Malthews, 1970; Ekici and Aytaç,2001). The genus is best represented in the steppe areas in the Irano-Turanian phytogeographic region of Turkey. The flowering plant of *Astragalus* genus, common name "milkvetches" or "locoweed" ("loco" is Spanish for crazy), was well known to western European botanists of the 17th century (Pistelli, 2002). The roots of *Astragalus* sp. have been shown to contain different types of secondary metabolites such as triterpene saponins, izoflavonoids, polysaccharides, amino acids, and trace elements (Juan et al., 2014; Jia et al., 2016; Zhang et al., 2016). Various biological activities have so far been reported on *Astragalus* species. The roots of Astragalus membranaceus, a well-known species, included in the official drug list of Traditional Chinese Pharmacopeia and prescribed mainly as tonic and for treatment of nephritis and diabetes (Pistelli, 2002). The compounds of this genus are used in the treatment of many diseases (Gökalp; 2020; 2021).

In vertebrates, the enzymes hydrolyse acetylcholine (ACh) exist in two diverse forms. Acetylcholinesterase enzyme (EC 3.1.1.7; AChE) terminates ACh activity at the post-synaptic membrane in the neuromuscular linkage (Aksu et al., 2016; Taslimi et al., 2017; Kocyigit et al., 2017). The other key enzyme hydrolyses ACh is in multitude in comparison to other esters but has no recorded physiological function. It is called nonspecific cholinesterase, cholinesterase (EC 3.1.1.8), butyrylcholinesterase, and pseudocholinesterase. In this study, it is called butyrylcholinesterase (BChE), while cholinesterases refer to both BChE and AChE. In vertebrate cells, both enzymes have key roles and they are inhibited by 10-5 with Meserine, a property which distinguishes them from nonspecific esterases. BChE and AChE enzymes can be specifically inhibited by N,N'-di-isopropylphosphorodiamidic anhydride and BW284C51 (Garibov et al., 2016). BChE is within the scope of attention of pharmacologists, because of the role of the enzyme in the succinylcholine hydrolysis (SCh), having a potential to have an a short-acting blocking on the ACh receptor. For specific patient groups, slow SCh hydrolyzation might result in prolonged apnea, possibly related to a genetic alteration in the BChE (Taslimi et al., 2017). It is not clear whether AChE enzyme within such locations as the membrane of red blood cell, early myotendinous bond, and migrating neurocrest cells plays a vital role (Çağlayan et al., 2019; Erdemir et al., 2019; Yamalı et al., 2020). It has been reported that in embryonic extension, a successive or organizing pattern of AChE and BChE gives rise to the suggestion of BChE functioning as an embryonic AChE (Caglayan et al., 2019; Taslimi et al., 2019). Protein sequencing, as well as the newly recorded cDNA clones and determined amino acid sequences for both enzymes allow a better evaluation of BChE and AChE (Sujayev et al.,2016; Topal et al., 2016; Öztaskın et al., 2017).

About 3-7% of the aggregate population of all humans can be categorized within the group of the people diagnosed with diabetes mellitus (DM), as the leading endocrine disease, leading to mortality and morbidity (Demir et al., 2019). Through the action of pancreatic α–amylase, it is possible to hydrolyze and absorb starch molecule as glucose in the small intestine by α–glucosidase enzymes (Taslimi et al., 2018; Demir et al., 2018). In addition to its utilization of multiple methods, α-glucosidase inhibitors (AGIs) can be grouped into the possible methods in treatment (Paslimi et al., 2017; Demir et al., 2020). The dietary carbohydrates as sucrose and maltose are taken by certain traits of α-glucosidases viz. sucrase, maltase, isomaltase, and glucoamylase existing within the intestines. As a result, inhibitory process by such enzymes has the potential to reduce the postprandial hyperglycemia and thus may be among the vital approaches in the treatment of DMs (Türker et al., 2017; Taslimi et al., 2018). The AGIs were extracted from the natural sources as microbes, nutrients, and plants (Choi et al., 2005; Inyushkina et al., 2007; Noh et al.,2011; Kanget al., 2012). In this study, the aim was to characterize the antioxidant and antimicrobial activities of ethanol extracts

prepared from aerial parts and roots of *Astragalus dumanii* and anti-cholinesterase and α-glucosidase inhibitory activity of only aerial part *extracts* of its.

MATERIALS and METHODS

Plant material and extraction

Aerial parts and roots of *Astragalus dumanii* (Fabaceae) were collected from Turkey: B6 square, Sivas-Kangal-Gürün road inter-section, 1560 m, 39°07'53.1" N; 37°14'32.9" E, and identified by Dr. Mehmet Tekin, Trakya University, Edirne. Dried specimens were deposited at the herbarium of Faculty of Science, Cumhuriyet University, under the collector number M. Tekin, 1494. The aerial parts and roots of *A. dumanii* were air dried at room temperature with shade and grounded with laboratory type grinder. 100g of powdered plant material (aerial parts and roots separately) macerated with aqueous ethanol (80: 20, v/v) for one day and filtered through filter paper. The filtrate was evaporated in vacuum (via Buchi rotary evaporator R-100). Ethanolic extract from aerial parts and roots afforded the yield of 28.15% and 16.18% (w/w), respectively. The resulting product was kept at -20ºC.

Antioxidant activity

Radical scavenging activity

The scavenging potential of ethanol extracts for 1,1-diphenyl-2-picryl-hydrazil (DPPH) radicals was determined by means of the altered approach developed by Sannigrahi et al (2010). 3 mL of test samples in various concentrations were blended with 1mL of 0.1mM DPPH solution in MeOH. The mixture was incubated at 25ºC. The absorbing status was determined at 517 nm utilizing a UV-VIS spectrophotometer after 30 min. The radical scavenging activity was determined through decreasing absorbance rate by the equation below:

% Scavenging activity = (Ac-As)/Ac × 100,

where As is the sample absorbing value, and Ac is the control absorbing value (without extract)

ABTS [2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)] radical cation scavenging potential

In measurement of radical scavenging potential of the ABTS cation, the specific method was utilized (Re et al., 1999). Interacting 7 mM ABTS solution with 2.45 mM potassium persulfate, subsequently keeping the mixture at 25ºC for 12–16 h in dark, ABTS radical cation was attained. As a preliminary step of the assay, ABTS working solution was attained by diluting the stock ABTS solution with methanol to give the absorbance of 0.700 ± 0.02 at 734 nm. 1mL of sample solution was blended with 1 mL of ABTS working solution. The absorbing capacity of resulting compost was ascertained at 734 nm following 7 min incubation period at room temperature. The results were expressed as IC_{50}.

Total phenolic content (TPC)

In line with the procedure described by Adedapo et al., 2008, TPC was detected with the Folin- Ciocalteu (F-C) approach. 1mL of test solutions with different concentrations and 4 mL of 7.5% Na_2CO_3 solution, and 5mL of 10% Folin- Ciocalteu reagent were mixed. After vortexing, the mixture was allowed to stand for 2h for color development. Then the absorbing capacity was evaluated at 760 nm with UV-VIS spectrophotometer. Expression of total phenolic content was determined as mg gallic acid equivalent per gram of extract.

Total flavonoid content (TFC)

TFC of the extracts was detected with aluminum chloride colorimetric method descried by Erygur et al. (Eruygur et al., 2017) with some modifications. On to the 0.5 mL of test sample (2 mg mL^{-1} in methanol) in test tubes, 4.3 mL of 80% ethanol, 0.1 mL of 1 M sodium acetate, and 0.1 mL of 10% aluminum chloride (w/v) were added. Following a 30min period left for incubating at 25ºC, the absorbing capacity was detected at 415 nm in comparison to the curve ofquercetin. The TFC was determined in mg of quercetin equivalents (QE) per gram of extract.

Antimicrobial activity

The minimum inhibitory concentration (MIC) of *A. dumanii* aerial parts and roots were determined using the broth microdilution method in 96-well microtiter plates (Eloff, 1998). The bacterial and yeast test strains used in this study were *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 29213), *Streptococcus pyogenes* (ATCC 19615), *Klebsiella pneumonia* (ATCC 13883), *Pseudomonas aeruginosa* (ATCC 27853), *Enterococcus faecalis* (ATCC 29212), *Candida albicans* (ATCC 10231) and *Candida tropicalis* (DSM 11953) (Eruygur et al., 2017). Mueller-Hinton broth (Accumix®AM1072) was used as a culture media for bacteria and Sabouraud Dextrose Broth (Himedia ME033) was used for *Candida spp.* (CLSI, 2002, CLSI, 2012).

The extracts were dissolved in the broth (512 mg mL^{-1}). 100 µL of the extract including broth was added to the first row of the microtiter plates. 50 µL of media was added the remaining wells and serial two-fold dilutions were prepared. The 11^{th} wells were used as the reproductive controls and 100 µL broth was added. The concentration of the extract in the wells was ranging from 256 to 0.5 mg mL^{-1}. The bacteria and fungi suspensions (50µL) were added to prepared samples. The final inoculum size was 5×10^5 CFU/mL in the bacteria wells and $0.5\text{-}2.5\times10^3$ CFU/mL in the

Candida sp. wells (CLSI, 2002, CLSI, 2012). The incubation of plates took place at 37°C for 24h for bacteria and at 30°C for yeasts. Then 2 mg mL^{-1} of 2, 3, 5-Triphenyltetrazolium chloride (TTC) sterile solution was blended and further incubated at 37°C for 1h (Eloff, 1998). The presence of a red pellet located at the well bottom with formation of formazan indicated microbial growth.

AChE/BChE activity determination

The inhibition potential of aerial parts *extracts* of *A. dumanii* on AChE/BChE were determined conforming to spectrophotometric method of Ellman et al.(Ellman et al., 1961). For substrates for the reactions mentioned above, acetylthiocholine and butyrylthiocholine iodides (AChI/BChI) were utilized. Namely, after dissolving through deionized water at different concentrations, 750 µL of solution, 50 µL BChE/AChE (5.32×10^{-3} U) solution and 100 µL of Tris/HCl buffer (1 M, pH 8.0) were mixed and incubated for 8 min at 20 ºC. Then, 50 µL of DTNB (0.5 mM) was added to the solution. For the activities of AChE/BChE, 5,5'-Dithio-bis(2-nitro-benzoic) acid (DTNB) acted as the measurement criteria. Addition of 50 µL of BChI/AChI initiated the reaction (Giacobini, 2003). The spectrophotometric monitoring of BChI/AChI substrates hydrolysis indicated that maximum absorption took place at 412 nm wavelength (Silman and Sussman, 2005).

α-glucosidase inhibition assay

In line with the procedure of Tao et al. (Tao et al., 2013) using p-nitrophenyl-D-glycopyranoside (pNPG) as the substrate, α-Glycosidase inhibiting potential for aerial parts *extracts* of *A. dumanii* was performed. 10 mg dissolved in 10 ml (EtOH:H_2O) was the procedure for sample preparation. As the preliminary step, in phosphate buffer (0.15 U mL^{-1}, pH 7.4) and 10-100 µL of the sample, 100 µL of phosphate buffer was blended with 20 µL of the enzyme solution. Multiple solutions in phosphate buffer were prepared in order to attain a complete inhibitory effect. Then, it was pre-incubated at 35 ºC for 12 min prior to the addition of the p-NPG to initiate reaction. 50 µL of p-NPG in phosphate buffer (5 mM, pH 7.4) was added after preincubation and re-incubated at 37 ºC. The absorbing capacities were spectrophotometrically measured at 405 nm. The IC_{50} amount was calculated from activity (%) versus plant concentration plots (Demir, 2019;, Demir, 2020).

Statistical analysis

The data were analysed by using MS Excel 2007 and presented as mean ± SD of three replicates. One-way analysis of variance (ANOVA) and Tukey tests were performed by using SPSS ((Version 20.0, SPSS Inc.,Chicago, IL, USA) to determine significant group differences and means were considered as statistically significant if $p < 0.05$.

RESULTS and DISCUSSION

Antioxidant activity

The present research focuses on finding naturally occurring natural antioxidants from plant origin. The results of different antioxidant assays on *A. dumanii* were showed in Table 1. Both of the radical scavenging activity was assessed by measuring the reduction in their absorbance at 734 nm for ABTS and 517 nm for DPPH. The DPPH radical are soluble in methanol while the ABTS is water soluble radical. As seen from the data given in Table 1, IC_{50} values for ABTS are lower than DPPH. It can be explained that the antioxidants present in the extract are relatively polar compounds (Soare et al., 2007). IC_{50} value for DPPH radical scavenging activity of ethanol extract from root (1008.88 µg mL^{-1}) was higher than aerial part (1398.08 µg mL^{-1}). On the other hand, ABTS radical scavenging activity of aerial part extract (1.18 µg mL^{-1}) higher than root extract (82.25 µg mL^{-1}).

Table 1. Radical scavenging activity (IC_{50} in µg ml^{-1}), total phenol and flavonoid content of ethanol extracts from aerial parts and roots of *A. dumanii* (values are given as mean ± SD of 3 measurements)

Tablo 1. A. dumanii'nin (sultan geveni) topraküstü ve köklerinden elde edilen etanol ekstrelerinin radikal süpürme aktivitesi (IC_{50} µg ml^{-1} cinsinden), toplam fenol ve flavonoit içeriği (değerler 3 ölçümün ortalama ± standard sapma (SD) olarak verilmiştir)

	Radical scavenging activities		Total phenolic content (mg GAE/g)	Total flavonoid content (mg QE/g)
	DPPH assay	ABTS assay		
*R*oot ethanol extract	1008.88 ± 0.04[a]	82.25 ± 0.01[a]	5.31 ± 0.03	8.26 ± 0.004
*A*erial part ethanol extract	1398.08 ± 0.03[b]	1.18 ± 0.002[b]	13.23 ± 0.05	7.93 ± 0.005
Gallic acid	5.59 ± 0.06[c]	56.01 ± 0.004[ab]		

Different letters in the same column were significantly different ($p < 0.05$).

Based on these results, it was suggested that the root of *A. dumanii* has a potential candidate for polar radical scavenger. Lim et al. reported that *A. sinicus* aceton extract showed strong DPPH radical scavenging activity than other extracts, showed 95.1% scavenging activity at 10 mg mL^{-1} concentration (Lim et al., 2011). Asgarpanah reported that the IC_{50} value of DPPH radical scavenging activity of methanol

extract from *A. squarrosus* is 1220 µg mL^{-1}(Asgarpanah et al., 2011). These results are in consistent with the results obtained in this study.

Phytochemical analysis revealed the presence of volatile oils, phenolic, flavonoids, sterols, and tannins in methanol extracts of *Astragalus* species. Plant phenolic constituents exhibit important biological activities. Phenolic compounds and flavonoids are known as potential antioxidants for their scavenging free radicals and chelating metal ions in the biological reaction chain. (Demir et al., 2017, Özaslan et al., 2018). Phenolics and flavonoids concentration in the extract are indicated as mg of gallic acid and mg of quercetin per g of extracts, respectively. The higher concentrations of polyphenolics were found in extract of aerial parts (13.23 ± 0.05 mg GAE g^{-1}) than roots (5.31 ± 0.03 mg GAE g^{-1}), whereas the content of flavonoids are higher in the roots (8.26 ± 0.004 mg QE g^{-1}) than aerial parts (7.93 ± 0.005 mg QE g^{-1}) and it is probably responsible for their free radical scavenging activity.

Antimicrobial activity

The antimicrobial activities against five bacteria and two yeast of the raw aqueous ethanol extract prepared from aerial parts and roots of *A. dumanii* were detected using the microdilution technique at the concentration range 32 to 256 mg mL^{-1} (Table 2).

Table 2. Antimicrobial activities of *A. dumanii* aerial parts and root extracts (MIC values, mg mL^{-1})
Tablo 2. A. dumanii toprak üstü ve kök ekstrelerinin antimikrobiyal aktiviteleri (MIC değerleri, mg mL^{-1})

	E. coli	*S. aureus*	*S. pyogenes*	*K. pneumoniae*	*P. aeruginosa*	*E. faecalis*	*C. albicans*	*C. tropicalis*
A. dumanii aerial part extract	256	32	128	256	128	32	32	256
A. dumanii root extract	256	128	256	256	256	64	64	256

According to Holetz et al. (2002) if the extracts displayed an MIC less than 100 µg mL^{-1}, the antimicrobial activity is good; from 100 to 500 µg mL^{-1} the antimicrobial activity is moderate; from 500 to 1000 µg mL^{-1} the antimicrobial activity is weak; over 1000 µg/ml the extract is considered inactive.

According to these criteria, the raw aqueous ethanol extracts of *A. dumanii* not showed antimicrobial activities on tested microorganisms.

Enzyme inhibition studies of the extract

Alzheimer (AD) is characterized as an advanced, neurodegenerative disease predominantly inflicting the people over 60 years of age and it is estimated to responsible for 50-60% of cases of dementia in humans within and above this age limit. The important symptoms associated with the later phases of AD involve cognitive dysfunction, primarily memory loss (Türkeş et al., 2019; Gündogğdu et al., 2019). In the brain of mammals, two main forms of ChEs exist, that is, AChE and BChE. Among the considerable biochemical changes in AD patients, diminution of ACh amounts in the cortex and hippocampus of the brain is the most noticeable (Işık et al., 2019). Thus, inhibition of AChE enzyme, hydrolyzing ACh at the cholinergic synapse is presently the most accepted approach to treatment of AD (Işık, 2019; Topal, 2019). The important side effects caused by licensed drugs utilized in treatment of AD have forced researchers to consider extracting safer BChE or AChE inhibitors from natural sources. Various plants and their constituents have long been benefited in traditional medicinal practices for improving cognitive function and alleviating other symptoms of AD, including depression (Öztaşkın et al., 2015;Istrefi et al., 2020).

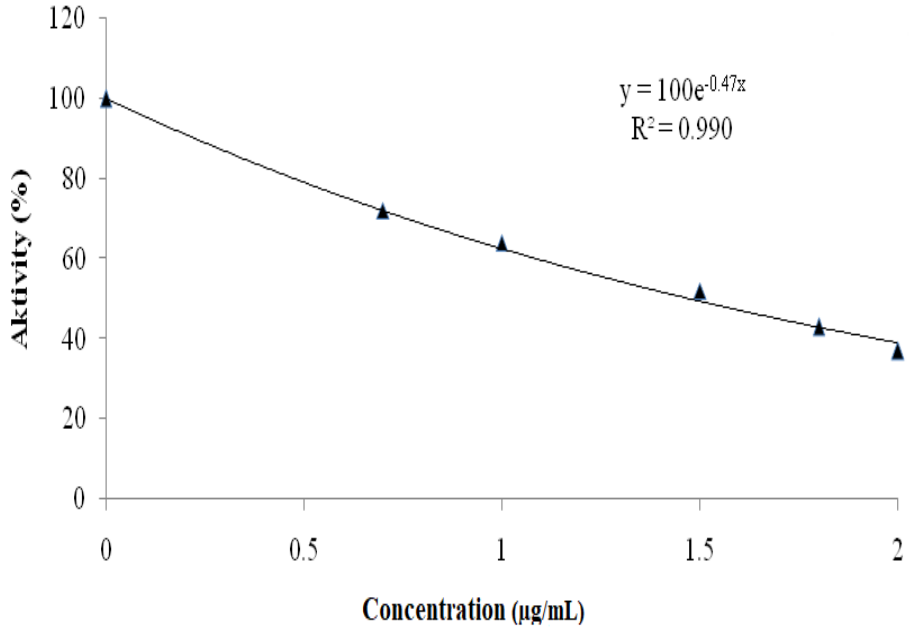
It is very important to discover new inhibitors that have less side effects than currently used cholinesterase inhibitor drugs, are cheaper and easily obtainable. Therefore, the discovery of new inhibitors is one aspect of this study. The effect of *A. dumanii aerial parts extracts* on enzyme activities was investigated spectrophotometrically under in vitro conditions and the results were compared with standard substances.

Aerial parts extracts of A.*dumanii* plant effectively resulted in the inhibition of AChE and BchE (Figure 1. and Table 3). TAC (9-amino-1,2,3,4-tetrahydroacridine) reversibly inhibits of BChE and AChE and it can be categorized as the first drug to be recommended for the placative treatment of AD. IC_{50} values for these enzymes were obtained 1.47 µg mL^{-1} (r^2 : 0.990) for AChE and 0.83 µg mL^{-1} (r^2 : 0.982) for BChE. Moreover, Tacrine (TAC) was used as positive standard BChE and AChE inhibitor with IC_{50} values 19.11 µg mL^{-1} (r^2 : 0.981) and 12.36 µg mL^{-1} (r^2 : 0.958) , respectively. These results showed us *Aerial parts extracts of* A.*dumanii* is a potential cholinesterase inhibitor.

Due to their modulatory physiological effects in the cure and prevention of obesity and diabetes, functional food profiles and plant-based medicines propelling a new surge of interest. Accordingly, the highly beneficial and attractive purposes as in vitro

inhibition of enzymes α-amylase and α-glucosidase are presently under wide scrutiny (Burmaoglu et al., 2019). Reports considering the safety and efficiency of natural nutritious supplements and specific herbs which have long been used for treating diabetic disease in traditional medicine and both academic and public interest in such reports have been accumulating (Gondolova et al., 2018).

In the current study, it was also studied inhibition effect of extract of A.dumanii on α-glycosidase. IC_{50} value was found 0.48 µg mL^{-1} (r^2 : 0.972) for α-glycosidase. The results demonstrate that *Aerial parts extracts of* A.dumanii inhibited α-glucosidase more effectively than acarbose, which had IC_{50} values of 22.80 (r^2 : 0.985)) µg mL^{-1} for a-glucosidase.



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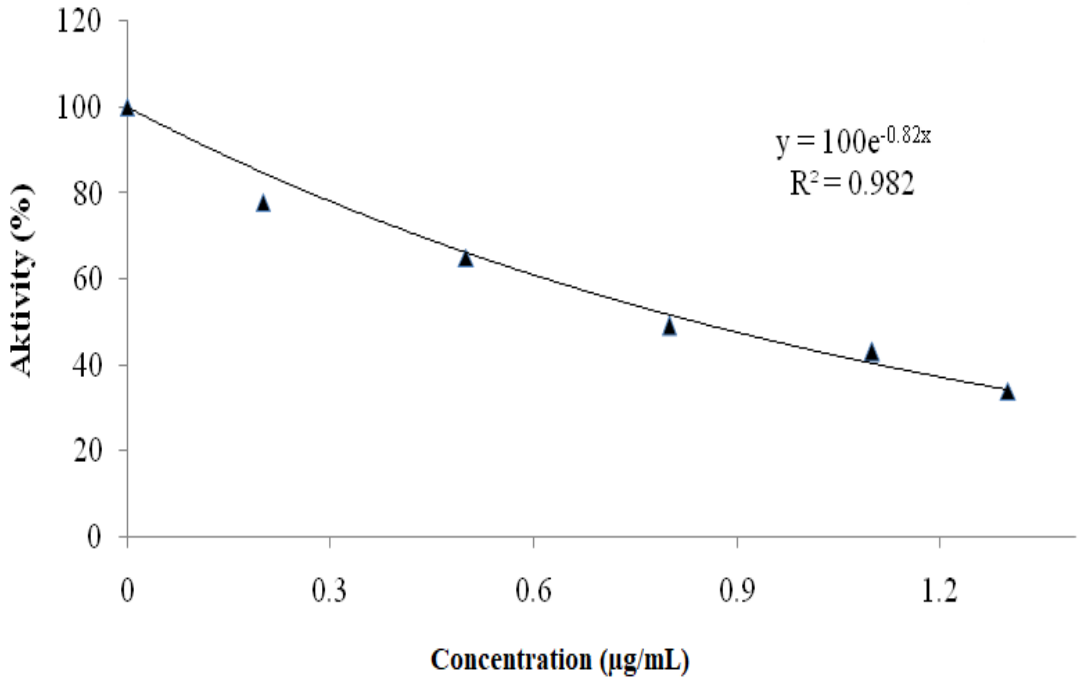

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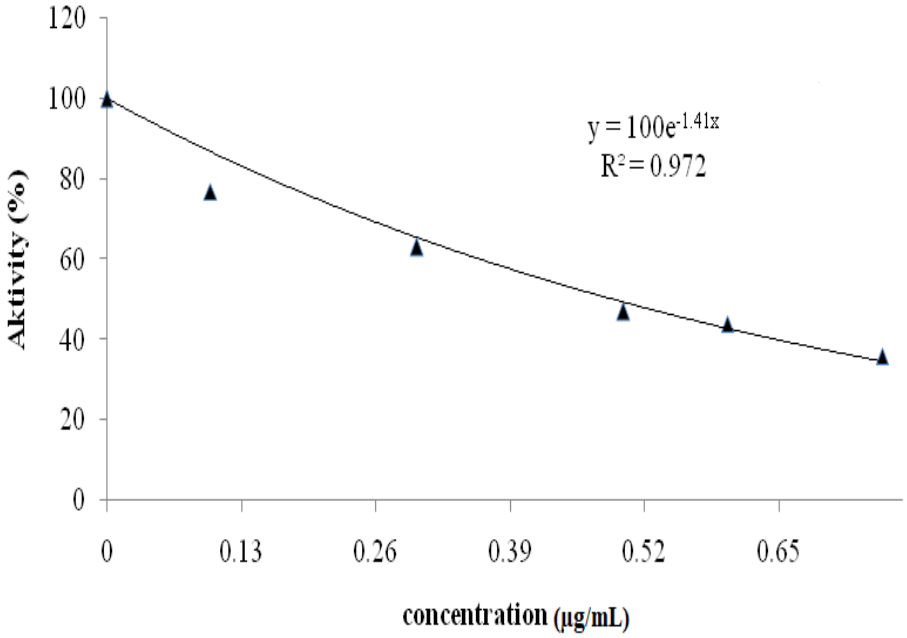

C

Figure 1. The IC_{50} graphs of aerial parts extracts of *A. dumanii* against A) AChE, B) BChE, C) α-glucosidase enzymes

Şekil 1. A. dumanii'nin (sultan geveni) toprak üstü kısım ekstresinin A) AChE, B) BChE, C) α-glukosidaz enzimlerine karşı IC_{50} grafikleri

Table 3. The enzyme inhibition results of aerial parts extracts of *A. dumanii* against AChE, BChE, and α-glycosidase enzymes.

Tablo 3. A. dumanii'nin (sultan geveni) toprak üstü kısım ekstrelerinin AChE, BChE ve α-glukosidaz enzimlerine karşı enzim inhibisyon sonuçları

	α-glucosidase		AChE		BChE	
Compounds	IC_{50} (µg mL^{1})	r^2	IC_{50} (µg mL^{-1})	r^2	IC_{50} (µg mL^{-1})	r^2
A. dumanii	0.48	0.972	1.47	0.990	0.83	0.982
Tacrine#	-	-	12.36	0.958	19.11	0.981
Acarbose*	22.8	0.985	-	-	-	-

#Tacrine was used as positive control for AChE and BChE enzymes and determined
*Acarbose was used as positive control for α-glycosidase enzymes and determined

CONCLUSION

Evaluation of anticholinergic, antidiabetic and antioxidant activity of *A. dumanii*, an endemic plant, is very important. Inhibition of active enzyme activity has become a prominent target in the therapy or management of many chronic disorders, particularly Alzheimer's disease, cancer, and diabetes. The results show that Astragalus dumanii is a natural source of antioxidants and is also a good enzyme inhibitory agent against acetylcholinesterase, butyrylcholinesterase and α-glycosidase (IC_{50} values: 1.47, 0.83 and 0.48 µg mL^{-1}, respectively) when compared with standard substances.In addition, the obtained results can provide a pharmacological basis for further research on the bioactivity isolation of active compounds.

CONFLICT of INTEREST

The authors report no declarations of interest.

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