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Kırktekesakalı (*Scorzonera pygmaea* Sibth.&Sm.) Bitkisinin In-Vitro Anti-Alzheimer Aktivitesi

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Özet

Scorzonera L. cinsinin 50'den fazla türü Türkiye'de doğal olarak yetişmektedir. Asteraceae familyasına ait bu cinsin üyeleri dünyanın birçok bölgesinde hem gıda hem de halk ilacı olarak kullanılmaktadır. Bu çalışmada Türkiye'nin endemik bitkilerinden olan *Scorzonera pygmaea* Sibth.&Sm. türünün anti-Alzheimer aktivitesi konu edilmiştir. Bitkinin kök (SPR) ve toprak üstü (SPH) kısımlarından elde edilen etanol ekstresi artan polariteye dayalı olarak sırasıyla petrol eteri (PE), kloroform (CH), etil asetat (EA) ve n-butanol (BU) ile tüketilmiştir. Elde edilen bu ekstrelerin in-vitro asetilkolin esteraz (AChE) ve butirilkolin esteraz (BChE) enzimlerini inhibisyonları Ellman yöntemi ile araştırılmıştır. Her iki enzim için pozitif standart olarak galantamin (G) kullanılmıştır. Hem AChE hem de BChE inhibisyonunda en potent ekstre sırasıyla % 59,72 (G: % 88,21) ve % 64,10 (G: % 81,80) inhibisyon ile SPR-CH olarak bulunmuştur. Ayrıca CH ekstreleri BChE'a karşı az da olsa daha spesifik iken kalan tüm ekstrelerin daha çok AChE'a karşı daha aktif olduğu gözlenmiştir. Bunlar içinde özellikle PE ekstrelerinin bu iki enzime karşı olan afinitelerindeki fark kayda değerdir. Ayrıca bitkiden yaklaşık 5 yıl önce elde edilmiş olan aynı ekstrelerin 4-8 °C'deki stabilitelerini test etmek için yeni hazırlanan ekstrelerle beraber eski ekstrelerin de AChE inhibisyonları ölçülmüştür. Yeni ekstrelerin aksine bunların hiçbirinin aktivite göstermediği belirlenmiştir. Bu bağlamda bitkinin kurutulmuş halde, oda sıcaklığında, nem, ısı ve ışıktan korunarak saklanmasının AChE inhibisyonu açısında daha uygun olduğu anlaşılmıştır. Sonuç olarak bitki orta derecede AChE, BChE inhibitör aktivitesine sahiptir ve bu etkilerden sorumlu bileşiklerin tespit edilmesi için CH ekstreleri üzerinde ileri fitokimyasal çalışmalara ihtiyaç duyulmaktadır.

Anahtar Kelimeler: *Scorzonera*, AChE, BChE, anti-Alzheimer

IN-VITRO ANTI-ALZHEIMER ACTIVITY OF KIRKTEKESAKALI (*Scorzonera pygmaea* Sibth.&Sm.)

ABSTRACT

More than 50 species of the genus *Scorzonera* L. grow naturally in Turkey. Members of this genus, belonging to the Asteraceae family, are used both as food and folk medicine in many regions of the world. In this study, anti-Alzheimer activity of *Scorzonera pygmaea* Sibth. & Sm., one of Turkey's endemic plant, has been subjected. Ethanol extract from root (SPR) and above ground-herba (SPH) parts of the plant were extracted with petroleum ether (PE), chloroform (CH), ethyl acetate (EA) and n-butanol (BU), respectively, based on increased polarity. In-vitro inhibition of acetylcholine esterase (AChE) and butyrylcholine esterase (BChE) enzymes of these extracts were investigated by Ellman method. Galantamine (G) was used as positive standard for both enzymes. The most potent extract in both AChE and BChE inhibition was SPR-CH with 59.72% (G: 88.21%) and 64.10% (G: 81.80%) inhibition, respectively. In addition, while the CH extracts were slightly more specific to BChE, it was observed that all the remaining extracts were more active against AChE. Among these, the differences in the affinities of the PE extracts to these two enzymes are particularly noteworthy. In addition, to test the stability of the same extracts stored at 4-8 °C, which were obtained from the plant about 5 years ago, AChE inhibition of the old extracts was measured together with the fresh prepared extracts. Unlike the fresh extracts, none of these were found to show activity. In this context, these results show that keeping the air-dried plant at room temperature by protecting from moisture, heat and light, is more suitable in terms of AChE inhibition. As a result, the plant has moderate AChE, BChE inhibitory activity and further phytochemical studies on CH extracts are needed to identify the compounds responsible for these effects.

Keywords: *Scorzonera*, AChE, BChE, anti-Alzheimer

INTRODUCTION

According to World Health Organization (WHO) approximately 50 million people worldwide have dementia, and it is predicted that this number will exceed 80 million by 2030. Although there are many types of dementia, the most common form is Alzheimer's disease (AD) and the prevalence of this disease is increasing significantly [1]. AD is a neurodegenerative disorder and its etiology is associated with abnormal accumulation of some peptides such as ß-amyloid plaques in nervous system [2]. Patients with AD are mostly characterised by loss of memory and impairment in the ability of daily physical, social activities such as speaking, learning etc [3]. None of the hypotheses proposed for the AD's treatment provide complete eradication, as the true cause and pathophysiology of this disease are still not fully understood. However, most of the current available therapeutics are based on the fact that the patients with AD have abnormal levels of cholinesterase (ChE) enzymes and this fact forms the "cholinergic hypothesis of AD". Cholinergic markers of the patients with AD support both this hypothesis and the development of ChE inhibitors which let choline to be available in the synapses for a longer time [4]. However, there are some reports to suggest that the role of ChE in AD not to be only about regulating levels of these enzymes in synaptic cleft[5]. Despite of its limitations such as providing only symptomatic treatment and being dependent on available receptors, ChE inhibitory activity is still a current subject in the studies about AD treatment / care.

It is inevitable to search for new active substances to be used in the treatment of diseases whose number and variety are increasing day by day. The majority of these researches consist of studies on natural origin materials and their synthetic / semi-synthetic derivatives. Therefore, it is of great interest to investigate the effects of plants against diseases, which are considered to be the largest of natural resources.

One of every ten flowering plants found on earth belongs to the Asteraceae family. The Asteraceae family is the most diverse family around the world, except Antarctica, with approximately 1,600-1,700 genera, 24,000-30,000 species, and nearly all kinds of habitats from plains to high mountains [6]. The genus

Scorzonera L. belonging to family Asteraceae, tribe Cichorieae, consist about 160 species from worldwide[7]. Most of the *Scorzonera* species are consumed as a vegetable and used as traditional medicine. There are more than 50 species growing in Turkey. These species are used against hypertension, rheumatism, kidney diseases, gout, diabetes, asthma, wounds, throat infection, stomach disorders, pain, infertility in Turkish folk medicine [8-10]. *Scorzonera pygmaea* Sibth. & Sm. is a perennial herb with pale yellow flowers and is endemic to Turkey [11,12]. Secondary metabolites including dihydroisocoumarins, isocoumarins, bibenzyls, phenolic acids and anti-inflammatory, antioxidant, antimicrobial activities of the plant were previously reported[13,14]. This study aims to investigate the anti-Alzheimer potential of the plant via acetylcholine esterase (AChE) and butyrylcholine esterase (BChE) inhibitory activity.

MATERIAL AND METHODS

Herbal Material

Flowering *Scorzonera pygmaea* Sibth.&Sm. were collected in July 2015 from Arayit Mountain (Eskişehir) at an altitude of 1830 m where its natural habitat. ESK 18397 is its voucher specimen code which was given by Osmangazi University Herbarium. The roots (radix) and above ground parts (herba) were separately airdried in the shadow. All material were stored by protecting light, moisture and heat at room temperature after grounded.

Extraction and Sample Preparation

40 g of the roots (SPR) were macerated with approximately 300 mL of ethanol for 24 hours. This step was repeated for 5 days. The ethanol extract was concentrated by using a rotary evaporator under reduced pressure at 45 °C. This extract was dissolved with a mixture of water : methanol (1:3 – 160 mL) and transferred to a 500 mL separating funnel. This hydro alcoholic mixture was extracted by adding and shaking 40 mL of petroleum ether (PE) for 3 times. PE soluble parts were collected and concentrated by using a rotary to provide PE extract. Then this process was repeated for chloroform (CH), ethyl acetate (EA) and n-butanol (BU) respectively. Eventually SPR-PE, SPR-CH, SPR-EA and SPR-BU extracts were prepared. All above mentioned steps were repeated for the above ground-herba (SPH) parts of the plant. Similarly, SPH-PE, SPH-CH, SPH-EA and SPH-BU extracts were obtained.

All extracts were evaporated to the dryness and stored at 4-8 °C until bioactivity studies. 4000 ppm (4 mg/mL) solutions in ethanol were prepared for each extract to test the anti-Alzheimer activity. After dilution with the solutions used in the activity methods, final concentrations of the extracts and standard were 200 μg/mL.

All solvents were analytical grade (Merck).

Anticholinesterase activity

A colorimetric and very common method reported by Ellman et al. was employed with minor changes to determine the inhibition of acetylcholine esterase (AChE) and butyrylcholine esterase (BChE) enzymes [15]. A phosphate buffer (pH: 8) was prepared with Na_2HPO_4 (disodium hydrogen phosphate), and NaH_2PO_4 (sodium dihydrogen phosphate) (Riedel-de Haën). Enzymes acetylcholinesterase (AChE), butyrylcholinesterase and their substrates acetylthiocholine iodide, butyrylthiocholine iodide were purchased from Sigma, Sigma, Applichem and Fluka respectively. 150 μL of phosphate buffer was transferred to all wells with 10 μL of 4000 ppm sample (extracts) solutions and 20 μL of enzyme solution (BChE or AChE). Plate was shaken and incubated at 25 °C for 15 minutes using a plate reader (BioTek Power Wave XS). After that 10 μL of 5,5-dithiobis (2-nitro benzoic acid) (DTNB) was added which is going to react with the final product (thiocholine) of the hydrolysis of the substrates by the enzymes. This reaction (after adding 10 μL of the either acetylthiocholine iodide or butyrylthiocholine iodide) results with yellow coloured 5-thio-2-nitrobenzoate whose absorbance was measured at 412 nm after 10 minutes. Three parallel reactions were carried out where galantamine (G) and ethanol were used as positive control and blank respectively. Results (% inhibition) were calculated according to following equation and given as mean ± standard deviation.

$$\text{Inhibition \%} = \frac{A_{blank} - A_{sample}}{A_{blank}} \times 100$$

RESULTS AND DISCUSSION

Amounts of PE, CH, EA, BU extracts obtained from the crude EtOH extracts of roots and above ground parts of *Scorzonera pygmaea* were given in Table 1.

Table 1: Extraction yields

	SPR (Roots)	**SPH** (Above ground)
EtOH	4.5 g	4.6 g
PE	487 mg	788 mg
CH	523 mg	477 mg
EA	297 mg	302 mg
BU	800 mg	828 mg

Results of anti-AChE and anti-BChE activities of the extracts were given in Table 2. Chloroform extract of the roots was the most potent extract against both AChE and BChE. However inhibitory activity of the chloroform extract of the above ground parts was not radically different from this extract for both enzymes. Compared to positive standard galantamine which is a dual, natural inhibitor of both enzymes, chloroform extracts have moderate anti-AChE and anti-BChE inhibitory potential. Secondary metabolites of this plant in terms of phenolic compounds were reported previously[13,14]. However, these studies focused on the EA extracts as the amount of total phenolics and the antioxidant capacity of the EA extracts were higher than the other extracts. This study showed that the chloroform extracts of the plant should be investigated to evaluate the plant about AChE, BChE inhibitory activities. Studies on anti-Alzheimer activity of the genus *Scorzonera* are limited. One of the most comprehensive study subjected 27 taxa of the genus *Scorzonera* but reported no significant AChE / BChE inhibitory activity[16]. However, studies conducted on *S. hieraciifolia*, *S. tomentosa*, *S. hispanica* and *S. papposa* are more promising and with the results of the current study encouraging to do more research on the subject [17-20].

Table 2: Results of in-vitro anti-Alzheimer activity of *Scorzonera pygmaea**

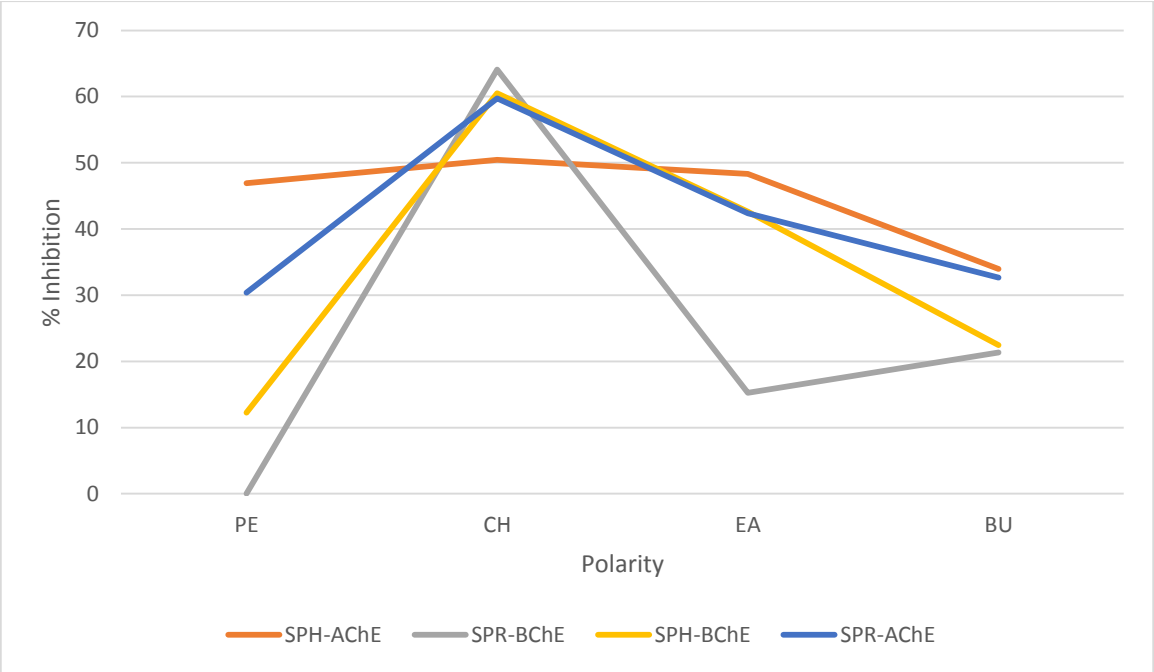
Samples	AChE	BChE
SPR-PE	30.36±0.52	NA
SPR-CH	59.72±1.35	64.10±1.22
SPR-EA	42.39±1.12	15.27±0.45
SPR-BU	32.66±0.57	21.34±0.31
SPH-PE	46.94±0.91	12.27±0.76
SPH-CH	50.47±0.54	60.49±0.73
SPH-EA	48.30±0.45	42.63±0.62
SPH-BU	33.97±0.50	22.44±0.83
Galantamine[a]	88.21±0.49	81.80±0.51

*****All enzyme inhibition values were given as inhibition % at 200 µg/mL
[a] Standard compound for AChE and BChE
NA:Not Active
SPR: *S. pygmaea* root, SPH: *S. pygmaea* above ground parts-herba, PE: Petroleum ether, CH: Chloroform, EA: ethyl acetate, BU: butanol

Another considerable result of this study is the different selectivity of the extracts against the enzymes. PE, EA and BU extracts but particularly PE extracts were selective to AChE while CH extracts slightly preferred BChE. Both enzymes present in the brain, but AChE predominates (% 80) in healthy brain. Thus, BChE is considered to have minor function in ACh levels. However, when it comes to brain with AD, while AChE levels are unchanged or declined, BChE levels significantly rise. This fact makes researchers think that selective BChE inhibitors may provide benefits in AD treatment[5,21-23]. In this regard, *S. pygmaea* extracts might be evaluated as a potential source for AChE selective, BChE selective and dual inhibitors.

Since extraction of the plant was made by partitioning, the changes in the inhibitory activities of the extracts by polarity were also examined (Figure 1). All extracts showed an increase in inhibitory potential (more or less) for both enzymes when shifting the polarity from petroleum ether to chloroform whereas a decrease was determined with changing the solvent to ethyl acetate from chloroform. This decrease continued with shifting the solvents to n-butanol (except SPREA in BChE inhibition). These results indicate that a solvent such as chloroform not nonpolar as much as petroleum ether and not polar as much as ethyl acetate might be appropriate to extract ideal compounds for AChE and BChE inhibitory activities.

Figure 1: The change of the inhibitory activities of the extracts by polarity



Finally, to test the stability of the same extracts stored at 4-8 °C, which were obtained from the plant about 5 years ago by the same method, AChE inhibition of the old extracts was measured together with the fresh prepared extracts. None of these old extracts were found to show any activity. This might be because of somehow deterioration of the compounds over a long period of time. In this context, these results show that keeping the air-dried plant at room temperature by protecting from moisture, heat and light, is more suitable in terms of AChE inhibition.

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DISCLOSURE STATEMENT

No potential conflict of interest was reported by the authors.

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