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ANTIOXIDANT, ANTICHOLINESTERASE INHIBITORY AND TYROSINASE INHIBITORY ACTIVITIES OF *Iris xanthospuria* EXTRACTS GROWING IN KÖYCEĞIZ REGION

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ABSTRACT

This study was aimed to investigate antioxidant, anticholinesterase inhibitory and tyrosinase inhibitory activities of *n*-Hex, DCM, EtOAc, and *n*-BuOH extracts obtained from rhizome and stem of *Iris xanthospuria*. Generally, it exhibited the best activity in both antioxidant activity tests of the rhizome and stem EtOAc extracts of the plant. EtOAc extracts reflected moderate activity on butyrylcholinesterase enzyme in BChE assay. EtOAc extract of rhizome showed the good tyrosinase inhibitory activity IC_{50} value of 4.30±0.25 g/L. In this study, EtOAc extracts may be potentially a source of natural antioxidant, anticholinesterase inhibitory and tyrosinase inhibitory activities.

Keywords: *Iris xanthospuria*, antioxidant activity, anticholinesterase inhibitory activity, tyrosinase inhibitory activity

INTRODUCTION

Plants -producers of various many novel bio-active molecules- are considered as the nature's medicinal factories. 80 % of the world population, mainly in the developing countries, still uses natural product-based traditional medicines [1].

Iris is a genus of Iridaceae contains perennial herbaceous plants that have been cultivated since ancient times as ornamental plants [2, 3]. In Turkey, Iris is named as 'süsen'; represented by 44

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taxa [3]. Iris species are fragrant, thus economically important in perfume and cosmetic industries. Most of its species have reported as anticancer, antiulcer, cytotoxic, insecticidal and piscicidal activities [2, 5, 6, 7, 8, 9, 11, 12, 13]. In Anatolian folk medicine, rhizomes of *I. germanica* and *I. pseudacarius* are applied to treat constipation and stomachache [14]. *I. xanthospuria* is an endemic perennial herb that blooms around Muğla and Hatay region of Turkey during April-May grown in.

The effect of oxidation on human health has been widely recognized. Oxidative metabolism is compulsory for the survival of cells. An antioxidant may be defined as: a substance that significantly delays or inhibits the oxidation of a substrate even at low concentrations, when compared with oxidizable [14, 15]. Antioxidants play an important role against the prevention and cure of various of chronic health problems such as Alzheimer's, ageing, cancer, and cardiovascular, atherosclerosis, stoke, diabetes, Alzheimer's diseases [16].

Free radicals are believed to contribute to ageing and age-related neurodegenerative disorders in the brain. The brain uses lots of oxygen to produce energy and these pruduce, it is also particularly susceptible to oxidative damage which has high levels of unsaturated fatty acids, which are particularly susceptible to damage, and relatively low levels of antioxidants. Oxidative damage in the brain triggers Alzheimer's disease. Brains of people being Alzheimer's disease are believed have excess levels of natural antioxidants responsible for removal of high levels free radicals, advising that the body is trying to struggle this damage [18].

Tyrosinase (EC 1.14.18.1), known as polyoxidase, monophenol oxidase, phenol or catecholase, is a monooxygenase enzyme cupper make to melanogenesis. Although it's founded as common in natural, generally are in fungal and some animal of microorganisms. Tyrosinase enzyme, specifically rises the concentration of melanine pigment affect to tyrosine substrate [19]. Over the past decade, strong tyrosinase inhibitors from natural and synthetic products are obtained [20, 21, 22, 23, 24]. Melanin is one of the factors affecting on skin and hair color in humans. It's necessary to protect human skin against radiation. To prevent pigmentation of abnormal melanin, side effects of prescription or non-prescription preparations require a minimum of new tyrosinase inhibitors.

On Iris species are present many biological activity studies as the antioxidant, anticholinesterase, antibacterial, immunomodulatory, anti-inflammatory, antifungal, and antiinsecticide. *I. albicans*

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exhibited the best antioxidant activity as *β*-carotene bleaching and CUPRAC assay and significantly against to butyrylcholinesterase inhibition activity assay from the chloroform extract of the rhizome of plant [25], the immunomodulatory activities of two isoflavones, 5,7-dihydroxy-6,4′-dimethoxyisoflavone possess stimulatory activity on T-cells and Th1 cytokine production, while 5,4′-dihydroxy-6,7-methylenedioxyisoflavone acted an immunosuppressant for T-lymphocytes (CD4+ and CD8+ cells) and cytokines isolated from *I. germanica* [26], *in vitro* biological activities including bactericidal, fungicidal and insecticidal activities as well as phytotoxicity and brine shrimp toxicity of the petroleum ether, chloroform and ethyl acetate extracts of *I. germanica* L.[2], antibacterial activity against *Acetobacter calcoacetica*, *Bacillus subtillis*, *Clostridium sporogenes*, *Clostridium perfringens*, *Escherichia coli*, *Salmonella typhii*, *Staphylococcus aureus*, and *Yersinia enterocolitica*. Its antifungal and antioxidant activities of the essential oil from the rhizomes of *I. bulleyana* [27].

Many phytonutrients or phytochemicals possess anticancer, antioxidant, anticholinesterase and tyrosinase properties. The presented study was aimed to evaluate the antioxidant, anticholinesterase inhibitory and tyrosinase inhibitory activities of *I. xanthospuria* extracts for the first time.

RESULT & DISCUSSION

The *in vitro* antioxidant, anticholinesterase inhibitory and tyrosinase inhibitory activity of eight extracts obtained from the rhizome and stem parts of *I. xanthospuria* collected from Köyceğiz-Turkey have reported with this study the first time. The differences observed in data in Table 1 and Table 2 thought to be due to their different phytochemical contents in extracts. The antioxidant activity results of *I. xanthospuria* given Table 1. In the 8 extracts, EtOAc extract in both the rhizome and stem seems to have the best antioxidant activity. Among the tested extracts, the EtOAc extract of rhizome (IC_{50}=14.80±0.69 mg/L) and the EtOAc extract of stem (IC_{50}=23.29±0.71 mg/L) showed the best cation radical scavenging activity. The same extracts exhibited as better activity IC_{50} value of 27.27±0.22 and 37.68±0.84 mg/L, respectively, than standard BHT (IC_{50}=54.97±0.99 µM) in DPPH free scavenging activity. According to the antioxidant activity assay results, the EtOAc extract of rhizome and stem demonstrated the best lipid peroxidation inhibitory activity IC_{50} value of 10.15±0.13 and 13.38±0.07 mg/L, respectively. The EtOAc extract

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of rhizome and the DCM, EtOAc, and *n*-BuOH extracts of stem indicated the best CUPRAC capacity activity with an $A_{0.5}$ value of 28.92±0.01, 25.59±0.01, 35.27±0.02, and 55.37±0.00 mg/L, respectively than α-tocopherol ($A_{0.5}$=40.55±0.04 mg/L) and BHA ($A_{0.5}$=32.71±0.02 mg/L) using as pharmaceutical standards. *I. albicans* reported that exhibited the best antioxidant activity as *β*-carotene bleaching and CUPRAC assay [25]. When compared to literature, it is obvious that the antioxidant activity of *I. xanthospuria* examined in this study are parallel to *I. albicans* in literature [25]. In this context, the EtOH extracts of *I. xanthospuria* rhizome and stem may use by people to protect against lipid peroxidation, free radical damage, and CUPRAC capacity, and its extracts will probably be used for the development of safe food products and additive substances.

Table 1. Antioxidant activity results of extracts of *I. xanthospuria*[a]

Sample	***Extract***	***Antioxidant Activity***			
		ABTS cation radical scavenging assay	***DPPH free radical scavenging assay***	***β-Carotene-linoleic acid assay***	***Cupric reducing antioxidant capacity***
		IC_{50} (mg/L)	***IC_{50} (mg/L)***	***IC_{50} (mg/L)***	***$A_{0,5}$ (mg/L)***
Rhizome	***n*-Hex**	73.29±0.35	67.75±0.11	59.20 ±0.48	64.46±0.00
	DCM	45.59±1.39	42.90±0.59	53.95 ±1.02	43.89±0.00
	EtOAc	14.80±0.69	27.27±0.22	10.15±0.13	28.92±0.01
	n-BuOH	28.96±0.37	39.05±0.39	32.66 ±0.91	51.70±0.00
Stem	***n*-Hex**	67.21±0.10	56.52±0.08	48.67±0.52	78.21±0.00
	DCM	27.19±0.53	41.31±0.57	39.75±0.69	25.59±0.01
	EtOAc	23.29±0.71	37.68±0.84	13.38±0.07	35.27±0.02
	n-BuOH	44.43±0.71	42.24±0.54	26.07±0.64	55.37±0.00
***α-Tocopherol*[b]**		4.87±0.45	12.26±0.07	4.50±0.09	40.55±0.04
***BHT*[b]**		2.91±0.55	54.97±0.99	2.34±0.09	4.00±0.04
***BHA*[b]**		4.10±0.06	19.40±0.47	1.34±0.04	35.71±0.02

[a]Value represent the means ± standard deviation of three parallel measurements (p<0.05)
[b]Reference compound

In view of the fact that products functioning as antioxidants can be used in the treatment of neuronal diseases [28], it demonstrated a good activity in both anticholinesterase activity tests of the both rhizome and stem EtOAc extracts of plant in general of this study (Table 2). EtOAc extracts of *I. xanthospuria* rhizome and stem showed significant activity against AChE IC_{50} value of 58.70±0.41 and 60.21±0.09 mg/L, respectively. EtOAc extracts of *I. xanthospuria* rhizome and stem showed significant activity against BChE showed the highest activity IC_{50} value of 53.25±0.88 and 56.77±0.80 mg/L, respectively. In a study, reported that exhibited significantly against to butyrylcholinesterase inhibition activity assay from the chloroform extract of the

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rhizome of *I. albicans* [18]. When compared to literature, it is obvious that the butyrylcholinesterase inhibition activity of *I. xanthospuria* examined in this study are parallel to *I. albicans* in literature [25]. In this context, EtOAc extracts of rhizome and stem may be potentially a source of natural anticholinesterases, and they could be converted into a usable product in pharmaceutical.

Tyrosinase inhibitors that have been an important role in the cosmetic and pharmaceutical industries for their skin-whitening effect sunburn, is used in the treatment of some dermatological disorders associated with melanin hyperpigmentation [28]. The tyrosinase inhibitory activity results of *I. xanthospuria* given Table 2. According to tyrosinase assay results of this study, which is the first tyrosinase inhibitory activity study of Iris species, EtOAc and DCM extracts of rhizome and stem showed the best tyrosinase inhibitory activity IC_{50} value of 4.30±0.25, 6.44±0.67, 6.11±0.40 and 8.72±0.66 g/L, respectively. In this context, it can be concluded that EtOAc and DCM extracts of rhizome and stem can be a potential candidate for the treatment of melanin biosynthesis related skin disease, likely hyper-pigmentation of human as well as animals.

Table 2. Anticholinesterase inhibitory and tyrosinase inhibitory activities of extracts of *I. xanthospuria*[a]

Sample	***Extract***	***Anticholinesterase inhibitory activity***		***Tyrosinase inhibitory activity***
		AChE assay IC_{50} (mg/L)	***BChE assay IC_{50} (mg/L)***	***Tyrosinase assay IC_{50} (g/L)***
Rhizome	***n*-Hex**	74.28±0.34	70.36±0.29	25.68±0.56
	DCM	70.42±0.73	66.75±0.43	6.44±0.67
	EtOAc	58.70±0.41	53.25±0.88	4.30±0.25
	n-BuOH	62.39±0.66	60.09±0.24	13.29±0.74
Stem	***n*-Hex**	79.08±0.62	76.58±0.17	26.06±0.78
	DCM	73.64±0.53	69.81±0.94	8.72±0.66
	EtOAc	60.21±0.09	56.77±0.80	6.11±0.40
	n-BuOH	65.70±0.97	64.42±0.33	16.35±0.52
Galantamine[b]		4.48±0.78	46.03±0.14	NT
Kojic acid[b]		NT	NT	0.64±0.12
L-mimosine[b]		NT	NT	0.67±0.06

[a]Value represent the means ± standard deviation of three parallel measurements (p<0.05)

[b]Reference compound

NT: Not tested

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EXPERIMENTAL

Chemicals and spectral measurements:

Ethanol, *n*-butanol, *n*-hexane, dichloromethane, chloroform, ethyl acetate, copper (II) chloride, sodium hydrogen phosphate, sodium dihydrogen phosphate, sodium hydrogen carbonate, potassium persulfate, ammonium acetate were obtained from E. Merck (Darmstadt, Germany). 2,2′-Azinobis (3-ethylbenzothiazoline-6-sulfonic acid)diammonium salt (ABTS), butyrylthiocholine iodide was obtained from FlukaChemie (FlukaChemie GmbH, Sternheim, Germany). *β*-carotene, linoleic acid, polyoxyethylenesorbitan monopalmitate (Tween-40), butylated hyrdoxyanisole (BHA), butylated hyrdoxytoluene (BHT), α-tocopherol, neocuproin, 1,1-diphenyl-2-picyrlhydrazyl (DPPH), acetylthiocholine iodide, 5,5-dithiobis-(2-nitro benzoic acid) (DTNB), galanthamine hydrobromide, 3,4-dihydroxy-L-phenylalanine (L-DOPA), acetylcholinesterase (AChE: from electric eel) (Type-VI-S, EC 3.1.1.7, 425.84 U/mg), and butyrylcholinesterase (BChE: from horse serum) (EC 3.1.1.8, 11.4 U/mg) were obtained from Sigma Chemical Co. (Sigma-Aldrich GmbH, Sternheim, Germany). All other chemicals and solvents used as analytical grade.

Bioactivity measurements were carried out on a 96-well microplate reader, SpectraMax 340PC^{384}, Molecular Devices (USA), at Department of Chemistry, Muğla Sıtkı Koçman University.

Plant material: *I. xanthospuria* rhizomes and stems were collected from Köyceğiz region of Muğla, Turkey, during April-May 2014, identified at the Herbarium of Biology, Faculty of Science, Muğla Sıtkı Koçman University, Turkey. The plant sample was confirmed by comparing it with the specimen voucher located at the stated herbarium.

Extracts Preparation: Dried rhizomes and stems were extracted by maceration with 80% ethanol, (20°C) for 24 h, concentrated under vacuum. Obtained residue was diluted with water, extracted with *n*-Hex, DCM, EtOAc and *n*-BuOH, respectively. All filtrations were dried by rotary evaporator.

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Determination of Antioxidant Activity

Solutions of extracts (*n*-Hex, DCM, EtOAc, *n*-BuOH) obtained from the rhizome and stem parts of *I. xanthospuria* were prepared at four different concentrations as 250-125-62,5-31,25 ppm in EtOH. EtOH was used as a control, while BHA and α-tocopherol were used as antioxidant standards for comparison of the activity tests. The results were given as 50% concentration (IC_{50}) for $ABTS^{\cdot+}$ scavenging activity, *β*-carotene-linoleic acid and $DPPH^{\cdot}$ assay while in the CUPRAC assay are expressed as $A_{0.5}$.

The spectrophometric analysis of antioxidant activities were performed according to the literature procedures as follows: $ABTS^{\cdot+}$ scavenging activity [29], *β*-carotene-linoleic acid [30,31], CUPRAC assay [32] and $DPPH^{\cdot}$ scavenging activity Blois [33].

Determination of acetylcholinesterase- (AChE) and butyrylcholinesterase- (BChE) Inhibitory Activity

AChE and BChE inhibitory activites were mesaured by the spectrophotometric method. Solutions of extracts (*n*-Hex, DCM, EtOAc, *n*-BuOH) obtained from the rhizome and stem parts of *I. xanthospuria* were prepared from the 250-125-62,5-31,25 ppm at concentrations. AChE from electric eel and BChE from horse serum were used, while acetylthiocholine iodide and butyrylthiocholine chloride were employed as substrates. DTNB (5,50-dithiobis(2-nitrobenzoic)acid was used for the measurement of the anticholinesterase activity [34].

Determination of Tyrosinase Inhibitory Activity

Tyrosinase inhibitory activity was determined by spectrophotometrically using mushroom tyrosinase according to Hearing method with slight modification by [35]. L -Dopa was used as substrate, and also kojic acid were used as standard inhibitors of tyrosinase. The percent inhibition of the enzyme and IC_{50} values of extracts were calculated using a program developed from the graph of tyrosinase inhibitory activity percentages (Inhibition %) against sample concentrations (µM).

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Statistical analysis

All data on biological activity tests were the averages of triplicate analyses. All biological activity tests were carried out at four concentrations, and the results are presented as 50% concentration (IC_{50}) and $A_{0.50}$ values. Data were recorded as mean ± SEM (standard error of the mean). Significant differences between means were determined by Student's-*t* test and *p* values <0.05 were regarded as significant.

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