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Screening antioxidant and anticholinesterase potential of *Iris albicans* extracts



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Abstract The antioxidant and anticholinesterase activities of the extracts prepared from the rhizomes and flowering aerial parts of *Iris albicans* were determined in this study. The chloroform extract of the rhizomes was rich in total phenolic contents ($431.98 \pm 0.49 \mu\text{gPEs/mg}$), and the chloroform extract of the aerial parts in total flavonoid contents ($663.05 \pm 0.32 \mu\text{gQEs/mg}$). Although the chloroform extract of the rhizomes exhibited the best antioxidant effect in β -carotene bleaching and CUPRAC methods among the tested extracts at all concentrations, it was found inactive in the metal chelating assay. The methanol extract of the aerial parts indicated moderate metal chelating activity (60%) at $100 \mu\text{g/mL}$. The chloroform extract of the rhizomes showed moderate anticholinesterase effect at $200 \mu\text{g/mL}$. The chloroform extract of the aerial parts showed significantly inhibition against butyrylcholinesterase ($78.44 \pm 0.51\%$).

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1. Introduction

Iris L. (Iridaceae) is widely distributed in Eurasia, North Africa, and North America (Rigano et al., 2009). This genus is represented by 46 taxa in Turkey, 16 of which are endemic (Mathew, 1984). Their rhizomes have been used as diuretics, carminatives and laxatives in Turkish folk medicine (Baytop,

1984). Some compounds and extracts of *Iris* species have been reported to possess anti-inflammatory, piscicidal, cytotoxic, hypolipidemic, antibacterial, antiulcer, antioxidant and anticholinesterase activities (Atta-ur-Rahman et al., 2003; Hacıbekiroğlu and Kolak, 2011). Besides their medicinal importance, they have an economic value, they have been used in perfume and cosmetic industries, and some of them have been cultivated as ornamental plants (Atta-ur-Rahman et al., 2004).

Iris albicans Lange is originating from Yemen and Arabia, frequently planted in many parts of the Mediterranean area (Williams et al., 1997). It grows in or near cultivated areas and cemeteries. Its leaves are markedly incurved at tips, strongly glaucous, its stem is usually simple with two terminal flowers which are normally white with a yellow beard on falls, rarely mid-blue. *I. albicans* Lange (cehennem zambağı, mezarlık zambağı in Turkish), known as Cemetery Iris,

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White Cemetery Iris, or the White Flag Iris, is a perennial herb and widespread in Turkey (Mathew, 1984). A literature survey showed that phytochemical and biological investigations on *I. albicans* were carried out by Williams et al. (1997) and Mothana et al. (2010). Mothana et al. also before determined the antimicrobial, antioxidant and cytotoxic activities of some Yemeni medicinal plants, and they reported that the methanolic extract of *I. albicans* possessed remarkable cytotoxic activity against FL-cells. In the same study, the methanolic and hot water extracts of *I. albicans* were found to be inactive in DPPH free radical scavenging activity method. The antioxidant and anticholinesterase activities of the fresh rhizomes and dried flowering aerial parts extracts of *I. albicans* were determined in this study.

2. Materials and methods

2.1. Plant material

The rhizomes and flowering aerial parts of *I. albicans* Lange (Syn: *Iris madonna*) were collected from Southern Turkey (Kozluca Köyü, Keçiborlu, Isparta) in May 2008, and identified by Dr. Necmettin Güler. A voucher specimen was deposited in the Herbarium of the Faculty of Science and Letters, Trakya University, Edirne, Turkey (EDTU 9626).

2.2. Preparation of the extracts

Fresh rhizomes of *I. albicans* (458.74 g) were cleaned with tap water, cut into pieces and ground in a grinder. They were sequentially macerated, for 24 h at room temperature, with petroleum ether (3×1.5 L), chloroform (3×1.5 L) and methanol (3×1.5 L). The flowering aerial parts were dried and powdered (1.1 kg), then sequentially macerated with petroleum ether (3×3 L), chloroform (3×3 L) and methanol (3×3 L). After filtration, the solvents were evaporated to dryness *in vacuo*. In Table 1, the yields of the extracts were given as w/w. After evaporation of the solvents, all crude extracts were directly used for the activity assays.

2.3. Determination of total phenolic content

The concentrations of phenolic content in the crude extracts were expressed as micrograms of pyrocatechol equivalents (PEs), determined with FCR according to the method of Slinkard and Singleton (Slinkard and Singleton, 1977). The solution of the crude extracts in methanol was added to 180 μ L of distilled water and 4 μ L of FCR (Folin–Ciocalteu's Reagent), and mixed thoroughly. After 3 min, 12 μ L sodium carbonate (2%) was added to the mixture and shaken intermittently for 2 h at room temperature. The absorbance was read at 760 nm. The concentration of phenolic

compounds was calculated according to the following equation (Absorbance = 0.0321 pyrocatechol (μ g) – 0.0457 ($R^2 = 0.9902$)).

2.4. Determination of total flavonoid content

Measurement of flavonoid content of the crude extracts was based on the method described by Park et al. (2008) with a slight modification and results were expressed as quercetin equivalents. An aliquot of the solution was added to test tubes containing 4 μ L of 10% aluminium nitrate, 4 μ L of 1 M potassium acetate and 172 μ L of methanol. After 40 min at room temperature, the absorbance was determined at 415 nm. The concentration of flavonoid compounds was calculated according to the following equation (Absorbance = 0.0751 quercetin (μ g) – 0.0652 ($R^2 = 0.9985$)).

2.5. Determination of the antioxidant activity with the β -carotene bleaching method

The antioxidant activity of the crude extracts was evaluated using the β -carotene-linoleic acid test system (Miller, 1971) with slight modifications. β -Carotene (1 mg) in 2 mL of chloroform was added to 100 μ L of linoleic acid, and 800 μ L of Tween-40 emulsifier mixture. After evaporation of chloroform under vacuum, 200 mL of distilled water saturated with oxygen, were added by vigorous shaking. Four thousand microliter of this mixture was transferred into different test tubes containing different concentrations of the sample. As soon as the emulsion was added to each tube, the zero time absorbance was measured at 470 nm using a 96-well microplate reader. The emulsion system was incubated for 2 h at 50 °C. A blank, devoid of β -carotene, was prepared for background subtraction. BHT and quercetin were used as standards.

2.6. Metal chelating activity

The chelating activity of the extracts on Fe^{2+} was measured as reported by Decker and Welch (1990). The extracts were added to a solution of 2 mM FeCl_2 (100 μ L). The reaction was initiated by the addition of 5 mM ferrozine (200 μ L). The mixture was shaken vigorously and left standing at room temperature for 10 min. After the mixture reached equilibrium, the absorbance was determined at 562 nm. EDTA was used as a reference compound.

2.7. Cupric reducing antioxidant capacity (CUPRAC)

Cupric reducing antioxidant capacity of the extracts was determined according to the method described by Apak et al. (2004). All crude extracts were dissolved in distilled water to prepare their stock solution at 1000 μ g/mL concentration. Sixty-one microliter of 1.0×10^{-2} M copper (II) chloride, 61 μ L of ammonium acetate buffer (1 M, pH 7.0), and 61 μ L of 7.5×10^{-3} M neocuproine solution were mixed, x μ L sample solution (2.5, 6.25, 12.5, and 25 μ L) and (67 – x) μ L distilled water were added to make the final volume 250 μ L. The tubes were stopped, and after 1 h, the absorbance at 450 nm was measured against a reagent blank, by using BioTek Power Wave XS.

Table 1 Yield of the extracts.

Yield (w/w %)			
Samples	Petroleum ether	Chloroform	Methanol
Rhizome	0.21	1.38	4.65
Aerial part	0.16	0.05	1.32

2.8. Anticholinesterase activity

Acetyl- and butyryl-cholinesterase inhibitory activities were measured by slightly modifying the spectrophotometric method developed by Ellman et al. (1961). Acetylthiocholine iodide and butyrylthiocholine iodide were used as substrates of the reaction and DTNB were used for the measurement of the anticholinesterase activity. All crude extracts were dissolved in ethanol to prepare their stock solution at 4000 µg/mL concentration. One hundred-fifty microliter of 100 mM sodium phosphate buffer (pH 8.0), 10 µL of sample solution and 20 µL AChE (or BChE) solution were mixed and incubated for 15 min at 25 °C, and 10 µL of DTNB is added. The reaction was then initiated by the addition of 10 µL acetylthiocholine iodide (or butyrylthiocholine iodide). Final concentration of the tested solutions was 200 µg/mL. The hydrolysis of these substrates was monitored using BioTek Power Wave XS by the formation of yellow 5-thio-2-nitrobenzoate anion as the result of the reaction of DTNB with thiocholine, released by the enzymatic hydrolysis of acetylthiocholine iodide or butyrylthiocholine iodide, at a wavelength of 412 nm. Ethanol was used as a solvent to dissolve the samples and controls.

3. Results and discussion

β -Carotene bleaching, metal chelating and CUPRAC assays were used to determine the antioxidant activity of the extracts prepared from the rhizomes and flowering aerial parts of *I. albicans* with their total phenolic and flavonoid contents as pyrocatechol and quercetin equivalents, respectively. Since the results of some studies showed that there are some relationship between antioxidant and anticholinesterase activities (Agrawal et al., 2009; Papandreou et al., 2009), the anticholinesterase activity was evaluated as well. This study provided the first antioxidant and anticholinesterase reports of *I. albicans* extracts. A literature survey showed that the anticholinesterase activity of only two *Iris* species, *Iris pseudopumila* and *Iris suaveolens*, had been investigated (Conforti et al., 2009; Hacıbekiroğlu and Kolak, 2011).

Flavonoids, especially isoflavones, and their glycosides, are the main constituents of *Iris* species (Al-Khalil et al., 1994). As shown in Table 2, the chloroform extract of *I. albicans* aerial parts was rich in flavonoid compounds (663.05 ± 0.32 µg) and the chloroform extract of the rhizomes in phenolic compounds (431.98 ± 0.49 µg).

The different methods with various mechanisms of reaction have been used to measure the antioxidant activity. Since the

results obtained from the different methods provide to determine the antioxidant potential of the tested samples, the activity was measured by three different methods in this study. Fig. 1 showed the inhibition of lipid peroxidation (total antioxidant activity) of the extracts determined by the β -carotene bleaching method. The results were compared with those of the standard compounds, quercetin (antioxidant of natural origin) and BHT (butylated hydroxytoluene, synthetic antioxidant). The total antioxidant activity increased with increasing amounts of the extracts. None of the extracts showed greater antioxidant activity than the standards. The chloroform extracts of the rhizomes and aerial parts indicated higher inhibition of lipid peroxidation than their petroleum ether and methanol extracts at all concentrations. The best inhibition (74%) was measured for the chloroform extract of the rhizomes at 80 µg/mL. The lipid peroxidation inhibition activity of the chloroform extracts may be related to the presence of the phenolic and flavonoid compounds.

The chelating activity for ferrous ion of the extracts was assayed by the inhibition of formation of red-colored ferrozine and ferrous complex. The methanol extract of the aerial parts exhibited the highest chelating activity among the tested extracts at 50 and 100 µg/mL (Fig. 2). EDTA was used as a standard compound, the methanol extract of the aerial parts showed moderate metal chelating activity (60%) at 100 µg/mL. The polar compounds may be responsible for this activity.

Cupric reducing antioxidant capacity method (CUPRAC) which was developed by Apak et al. is one of the electron-transfer based methods (Apak et al., 2004). As determined in the β -carotene bleaching method, the chloroform extracts of the rhizomes and aerial parts of *I. albicans* indicated the best cupric reducing power among the tested extracts (Fig. 3). The cupric reducing activity of the chloroform extract of the rhizomes was found to be higher than the petroleum ether and methanol extracts but lower than quercetin and BHT at all concentrations.

In this study, the antioxidant capacity of the crude extracts was compared with their anticholinesterase activity. Some clinical effects of medicinal plants are closely related to their antioxidant activity (Gu and Weng, 2001). However, the use of antioxidants may be relevant in slowing Alzheimer's disease progression and minimizing neuronal degeneration (Howes et al., 2003). Alzheimer's disease, being one of the neurodegenerative diseases, is an important health problem for elderly people. The anticholinesterase activity of the crude extracts was compared with galanthamine at 200 µg/mL. As shown in Table 3, the chloroform extract of the rhizomes possessed moderate anticholinesterase activity.

Table 2 Total phenolic and flavonoid contents of the extracts^a.

Extracts	Phenolic content (µg PEs/mg extract) ^b	Flavonoid content (µg QEs/mg extract) ^c
Rhizomes petroleum ether	139.61 ± 0.79	27.16 ± 0.34
Rhizomes chloroform	431.98 ± 0.49	174.34 ± 1.37
Rhizomes methanol	335.25 ± 0.87	104.61 ± 1.40
Aerial parts petroleum ether	135.92 ± 0.83	103.48 ± 0.56
Aerial parts chloroform	265.31 ± 1.37	663.05 ± 0.32
Aerial parts methanol	261.73 ± 1.84	259.94 ± 1.16

^a Values expressed are mean ± SD of three parallel measurements ($p < 0.05$).

^b PEs, pyrocatechol equivalents.

^c QEs, quercetin equivalents.

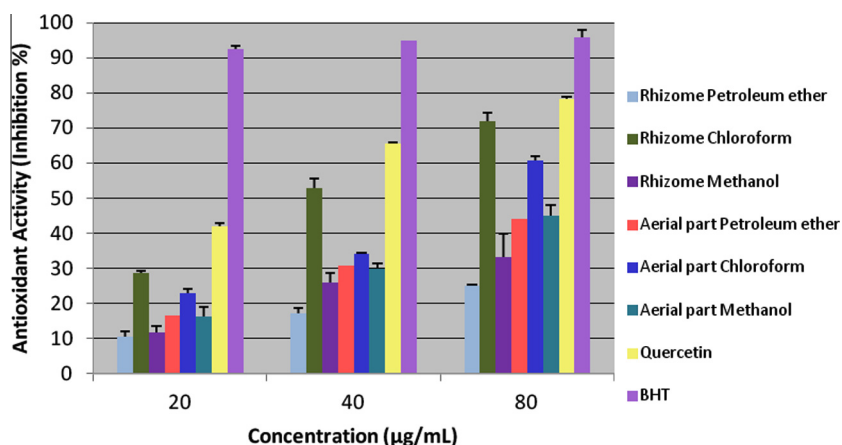


Figure 1 Inhibition (%) of lipid peroxidation of the extracts, quercetin and BHT by β -carotene bleaching method. Values are mean $\pm$ SD, $n = 3$, $p < 0.05$, significantly different with Student's t -test.

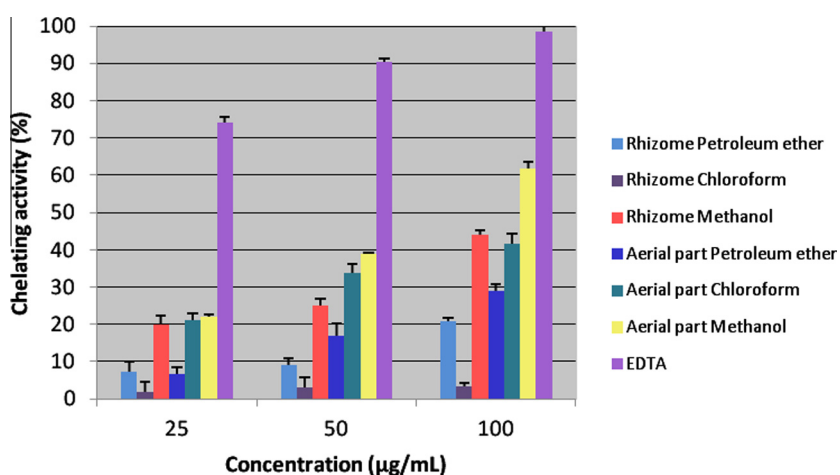


Figure 2 Chelating activity of the extracts and EDTA. Values are mean $\pm$ SD, $n = 3$, $p < 0.05$, significantly different with Student's t -test.

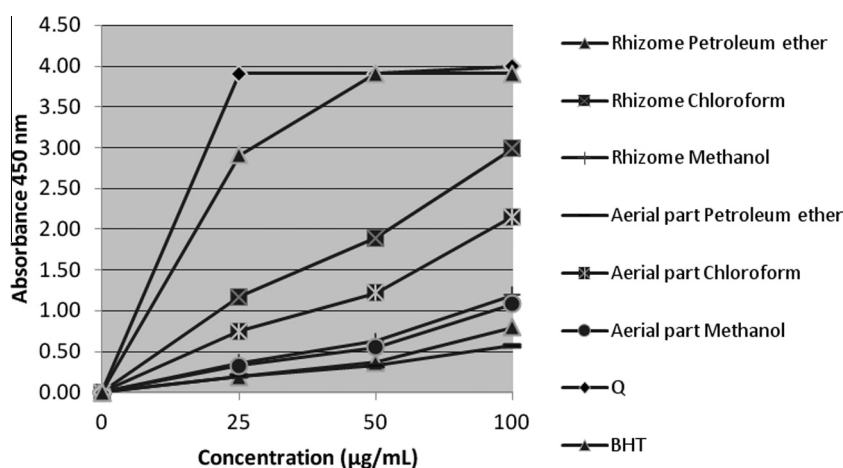


Figure 3 Cupric reducing antioxidant capacity of the extracts, quercetin and BHT. Values are mean $\pm$ SD, $n = 3$, $p < 0.05$, significantly different with Student's t -test.

Table 3 Anticholinesterase activity of the extracts at 200 µg/mL^a.

Extracts	Inhibition % against AChE	Inhibition % against BChE
Rhizome petroleum ether	NA	16.71 ± 1.72
Rhizome chloroform	44.50 ± 2.63	68.77 ± 1.00
Rhizome methanol	NA	1.85 ± 0.71
Aerial part petroleum ether	NA	44.10 ± 1.29
Aerial part chloroform	NA	78.44 ± 0.51
Aerial part methanol	NA	34.80 ± 0.72
Gаланthamine ^b	89.99 ± 0.11	87.17 ± 0.83

NA, not active at 200 µg/mL.

^a Values expressed are mean ± SD of three parallel measurements ($p < 0.05$).

^b Standard drug.

Although the chloroform extract of the aerial parts was found to be inactive against acetylcholinesterase, it exhibited strong inhibition against butyrylcholinesterase ($78.44 \pm 0.51\%$).

4. Conclusions

In conclusion, the chloroform extract of the rhizomes of *I. albicans* exhibited the best antioxidant and anticholinesterase activities among the tested extracts. This study indicated that there was a relationship between the antioxidant and anticholinesterase effects. This relationship was also determined in our previous study on the antioxidant potential and anticholinesterase activity of eleven edible plants. In that study, the ethanol extract of cinnamon possessed both strong CUPRAC and anticholinesterase activity (Boğa et al., 2011). Phytochemical studies are needed to determine the active constituents of the chloroform extract of *I. albicans*. Further *in vitro* and *in vivo* investigations will provide to indicate the antioxidant and anticholinesterase potential of the chloroform extract of *I. albicans* and its active compounds.

5. Statistical analysis

The results were mean ± SD of three parallel measurements. All statistical comparisons were made by means of Student's *t*-test, p values < 0.05 were regarded as significant.

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