PHYTOTHERAPY RESEARCH
Phytother. Res. **25**: 522–529 (2011)
Published online 9 September 2010 in Wiley Online Library
(wileyonlinelibrary.com) **DOI**: 10.1002/ptr.3299

Antioxidant and Anticholinesterase Constituents from the Petroleum Ether and Chloroform Extracts of *Iris suaveolens*

Işıl Hacıbekiroğlu and Ufuk Kolak*
Department of General and Analytical Chemistry, Faculty of Pharmacy, Istanbul University, 34116, Istanbul, Turkey

The aim of this study was to investigate the phytochemical, antioxidant and anticholinesterase activities of *Iris suaveolens*. After determining total phenolic and flavonoid contents of the petroleum ether, chloroform and methanol extracts prepared from the rhizomes, the antioxidant capacity of the extracts was established using β-carotene–linoleic acid and CUPRAC methods. The chloroform extract which was rich in phenolic content exhibited the highest inhibition of lipid peroxidation in the β-carotene–linoleic acid system, and the best cupric reducing antioxidant capacity among the tested extracts. The petroleum ether extract indicated moderate anticholinesterase activity while the chloroform extract revealed significant butyrylcholinesterase inhibitory activity (75.03 ± 1.29%). Spectroscopic methods were used for the structural elucidation of the compounds (1–13) isolated from the petroleum ether and chloroform extracts. Coniferaldehyde (6), having the highest antioxidant activity in the β-carotene–linoleic acid assay at 25 and 50 μg/mL, demonstrated also the best effect in the CUPRAC method among the tested compounds (1–12). 3-Hydroxyirisquinone (10) showed the best anticholinesterase activity among the tested compounds (1–4, 6–12), and coniferaldehyde exhibited almost the same butyrylcholinesterase inhibitory activity (82.60 ± 2.33%) as galantamine (86.26 ± 0.66%). Copyright © 2010 John Wiley & Sons, Ltd.

Keywords: Iris suaveolens; Iridaceae; coniferaldehyde; 3-hydroxyirisquinone; antioxidant; anticholinesterase.

INTRODUCTION

Iris L., comprising over 300 species, is a member of the Iridaceae family. *Iris* species have been used in the treatment of cancer, inflammation, bacterial and viral infections. Some *Iris* species have been cultivated as ornamental plants because of their coloured flowers, and they have been used in perfume and cosmetic industries due to their sweet fragrance due to iridal-type compounds (Atta-ur-Rahman *et al*., 2004). Isoflavones, flavones and their glycosides are the main classes of compounds found in *Iris* species. Iridals, flavanones, xanthones, stilbenes, quinones and peltogynoids have been also isolated from these species (Marner *et al*., 1995; Farag *et al*., 2009). Many of these compounds have been shown to possess piscicidal, antineoplastic, antioxidant, antitumour, antiplasmodial and antituberculosis properties (Atta-ur-Rahman *et al*., 2004).

There are 46 *Iris* L. taxons in Turkey, 16 of which are endemic (Mathew, 1984). The rhizomes of *Iris* species, known as 'süsen, navruz' in Turkey, have been used as diuretics, carminatives and laxatives in traditional Turkish medicine (Baytop, 1984). *Iris suaveolens* Boiss. & Reuter, a small perennial herb mainly distributed in the Balkans and Northwestern Turkey, has purple, yellow or bicoloured flowers with a purplish or brownish lamina to falls (the three sepals of *Iris* species, which are spreading or droop downwards, are referred to as 'falls') and with a yellow or bluish beard (Mathew, 1984). Although many contributions concerning the chemistry and activity of *Iris* species have appeared, there have been no reports on the phytochemical and activity studies of *I. suaveolens* (Marner *et al*., 1982; Atta-ur-Rahman *et al*., 2003).

In this study, the petroleum ether, chloroform and methanol extracts were prepared from the fresh rhizomes of *I. suaveolens*. The total phenolic and flavonoid contents of these crude extracts were determined as pyrocatechol and quercetin equivalents, respectively. The antioxidant and anticholinesterase activity tests of these crude extracts proved that the petroleum ether and chloroform extracts possessed significant antioxidant and anticholinesterase effects. Therefore the petroleum ether and chloroform extracts were separated using chromatographic methods to isolate their bioactive compounds. The structures of the isolated compounds **(1–13)** were determined on the basis of spectroscopic evidence (UV, ^{1}H- and ^{13}C-NMR, Mass). Five of which were isoflavones (tectorigenin **(1)** (Hanawa *et al*., 1991) irilone **(2)** (Al-Khalil *et al*., 1994), nigricin **(3)** (Al-Khalil *et al*., 1994), iriskumaonin methyl ether **(4)** (Veitch *et al*., 2003), soforanarin A **(5)** (Atta-ur-Rahman *et al*., 2004)), four phenolics (coniferaldehyde **(6)** (Sy and Brown, 1999), *cis*-epoxyconiferyl alcohol **(7)** (Guz and Stermitz, 2000), acetovanillone **(8)** (Crestini and D'Auria, 1997), *p*-hydroxyacetophenone

* Correspondence to: Ufuk Kolak, Department of General and Analytical Chemistry, Faculty of Pharmacy, Istanbul University, 34116, Istanbul, Turkey.
E-mail: ufukkolak@yahoo.com

Received 19 November 2009
Revised 19 July 2010
Accepted 03 August 2010

(9) (Senatore *et al*., 1999)), a quinone (3-hydroxyirisquinone **(10)** (Seki *et al*., 1995)), two steroids (7β-hydroxystigmast-4-en-3-one **(11)** (Carcache-Blanco *et al*., 2006), β-sitosterol **(12)** (Kolak *et al*., 2005)) and a fatty acid (*cis*-vaccenic acid **(13)** (Duffy *et al*., 2006)). The chemical structures of these known compounds **(1–13)** are presented in Fig. 1. All crude extracts and the compounds **(1–12)** were screened for their antioxidant activity using β-carotene–linoleic acid and CUPRAC methods, with quercetin and butylated hydroxytoluene (BHT) as positive controls. The acetyl- (AChE) and butyryl-cholinesterase (BChE) inhibitory activity of the crude extracts and the compounds **(1–4, 6–12)** was established using the Ellman method (Ellman *et al*., 1961).

MATERIALS AND METHODS

Plant material. The rhizomes of *Iris suaveolens* Boiss. & Reuter (Syn: *Iris mellita* Janka) were collected from Northwestern Turkey (Lalapaşa-Edirne) in April 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 9625).

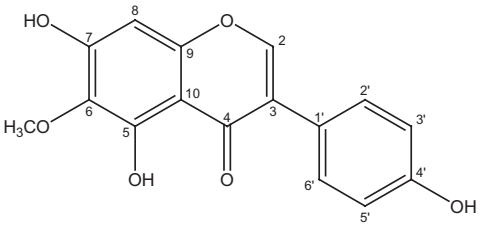
Chemicals. Silica gel 60 (1.07734), preparative TLC plates (1.05554), ethanol, toluene, hexane, chloroform, dichloromethane, diethyl ether, HPLC grade methanol, potassium acetate, butylated hydroxytoluene, cerium (IV) sulphate tetrahydrate, aluminium nitrate nonahydrate, sulphuric acid, aluminium chloride, sodium acetate and boric acid were purchased from Merck (Darmstadt, Germany), β-carotene, linoleic acid, Tween 40, copper (II) chloride dihydrate, quercetin, pyrocathecol, fenazin metasulphate, sodium methoxide, DTNB (5,5-dithiobis-(2-nitro benzoic acid)), acetylcholinesterase, butyrylcholinesterase from Sigma (Steinheim, Germany), acetylthiocholine iodide, Folin Ciocalteu Phenol reagent from Applichem (Darmstadt, Germany), α-tocopherol from Aldrich (Steinheim, Germany), neocuproine, galantamine hydrobromide from Sigma-Aldrich (Steinheim, Germany), petroleum ether, methanol, acetone, ammonium acetate, sodium carbonate, sodium hydrogen phosphate, sodium dihydrogen phosphate from Reidel de Haen (Germany), butyrylthiocholine iodide from Fluka (Steinheim, Germany).

General experimental procedures. The UV spectra (λ_{max}) were recorded on a Shimadzu UV-1601 in MeOH, NMR spectra on a Varian UNITY INOVA (^{1}H: 500 MHz, ^{13}C: 125 MHz), Bruker DRX 500 MHz (^{1}H: 500 MHz, ^{13}C: 125 MHz), and DRX 600 MHz (^{1}H: 600 MHz, ^{13}C: 150 MHz), and HRESI-MS spectra on a Bruker Microsoft Q spectrometer. LC-MS (ThermoFinnigan LCQ, column: Lichrospher 100 RP_{18} (5 μm). Solvent: MeOH:H_2O 20:80, flow: 1 mL/min, equipped with Hewlett Packard 1100), MPLC (Büchi 681 pump, column: C_{18}. Solvent: MeOH:H_2O 20:80, flow: 8 mL/min, Knauer UV detector), preparative HPLC (Knauer model K-1800 preparative pump, column: VP 250/10 Nucleodur C_{18} Pyramid (5 μm). Solvent: MeOH:H_2O 20:80, flow: 4 mL/min, Knauer UV detector), and analytical HPLC (Kontron model Analytic 410, column: VP 250/10 Nucleodur C_{18} Pyramid (5 μm). Solvent: MeOH:H_2O 20:80, flow: 2 mL/min, Knauer UV detector) were used for some chromatographic separations. A Thermo pH-meter, an Elma S15 ultrasonic bath, a vortex (LMS Co. Ltd), and a BioTek Power Wave XS were used for the activity assays.

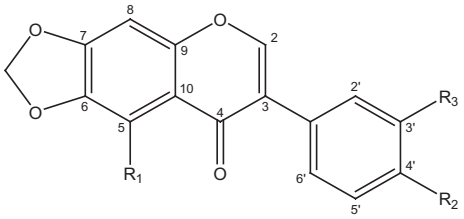
Extraction and isolation. Fresh rhizomes of *I. suaveolens* (1 kg) were cleaned with tap water, cut into pieces and ground in a grinder. They were sequentially macerated, for 24 h at room temperature, with petroleum ether (3 × 3 L), chloroform (3 × 3 L) and methanol (3 × 3 L). After filtration, the solvents were evaporated to dryness *in vacuo*. The petroleum ether extract (ISUP) (2.7 g) was subjected to a silica gel column (1.5 × 40 cm) and eluted with petroleum ether (40–60°) (5 × 150 mL), a gradient of chloroform was added in 10 mL increments into 100 mL petroleum ether until reaching 100% thus 10 × 100 mL were used, followed by methanol in 10 mL increments up to 100% (10 × 100 mL). Similar fractions were combined by using TLC analysis. Final purifications were carried out on preparative TLC plates using the following solvent systems for acetovanillone (**8**) (10 mg) (toluene: acetone:diethyl ether, 9:1:0.5), 3-hydroxyirisquinone (**10**) (24 mg) (toluene:acetone:diethyl ether, 8:0.5:0.5), 7β-hydroxystigmast-4-en-3-one (**11**) (1.5 mg) (toluene:acetone:diethyl ether, 6:0.5:0.5), β-sitosterol (**12**) (4.5 mg) (toluene.acetone:diethyl ether, 8:0.5:0.5), *cis*-vaccenic acid (**13**) (1.6 mg) (toluene:acetone:diethyl ether, 6:0.5:0.5). TLC plates were visualized by spraying with cerium (IV) sulphate dissolved in 10% sulphuric acid following UV light checking.

The chloroform extract (ISUC) (14 g) was fractionated on a silica gel column (2.5 × 150 cm). The column was eluted with petroleum ether, followed by a gradient of chloroform up to 100%, and then a 2% gradient of methanol up to 100%. The thin layer chromatography analysis was used to combine similar fractions, and ten fractions (fractions **A**–**J**) were obtained. A preliminary separation of fraction **D** (2.6 g) was carried out by MPLC on C_{18} column using a stepwise gradient of MeOH:H_2O (30:70, 40:60, 50:50, 60:40, 70:30, 80:20, 90:10, 95:5, 100:0, v/v, 200 mL each) into eight fractions. Fractions **D-3** and **D-5** were separated by using analytical and preparative HPLC, respectively, to afford tectorigenin (**1**) (12 mg), iriskumaonin methyl ether (**4**) (30 mg), coniferaldehyde (**6**) (4 mg) and *cis*-epoxyconiferyl alcohol (**7**) (5 mg) (see above for conditions). Fraction **F** (3 g) was separated by MPLC using C_{18} column (MeOH:H_2O, 40:60, 50:50, 60:40, 70:30, 80:20, 90:10, 95:5, 100:0, v/v, 200 mL each). The first separation of Fraction **F-10** (90 mg) was carried out by using analytical and preparative HPLC, respectively (see above for conditions), and the final purification was achieved on preparative TLC plates using the following solvent systems to give nigricin (**3**) (10 mg) (toluene:diethyl ether, 4:1), soforanarin A (**5**) (2 mg) (toluene:diethyl ether, 4:1), *p*-hydroxyacetophenone (**9**) (12 mg) (toluene:diethyl ether, 4:1). Fraction **G** (260 mg) was purified by preparative HPLC on a Nucleodur C_{18} Pyramid using MeOH:H_2O (20:80) to yield irilone (**2**) (24 mg).

*Tectorigenin (**1**)*. Pale yellow powder; UV λ_{max} (MeOH) nm: 214, 265; (MeOH + NaOMe) nm: 217, 278, 328 (sh); (MeOH + $AlCl_3$) nm: 210, 275; (MeOH + $AlCl_3$ + HCl) nm: 210, 277, 313 (sh); (MeOH + NaOAc) nm:



1

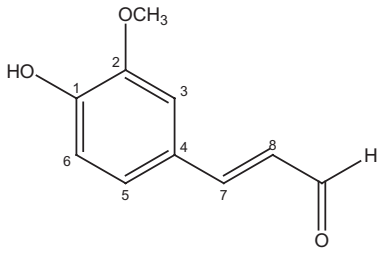


2 R_1= OH, R_2= OH, R_3= H

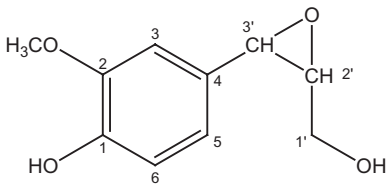
3 R_1= OCH_3, R_2= OH, R_3= H

4 R_1= R_2= R_3=OCH_3

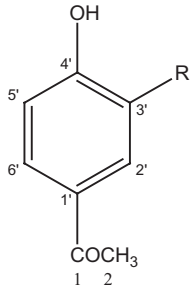
5 R_1= OH, R_2=OCH_3, R_3= OH



6

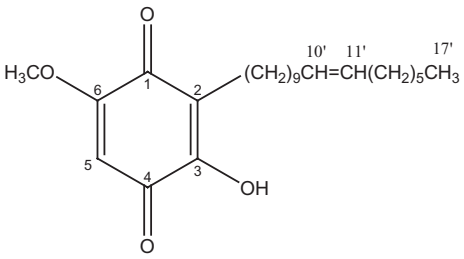


7

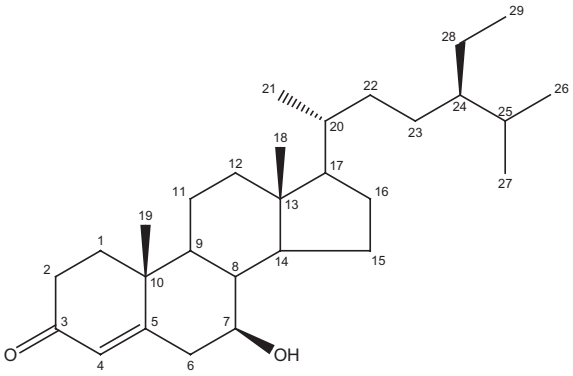


8 R= OCH_3

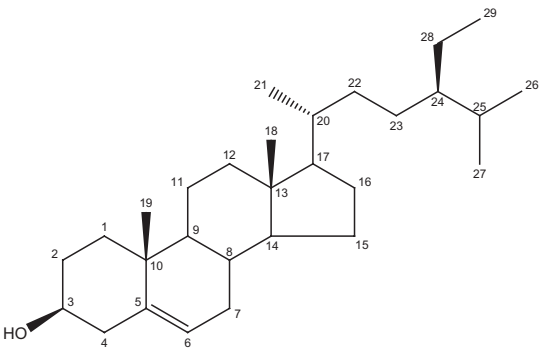
9 R=H



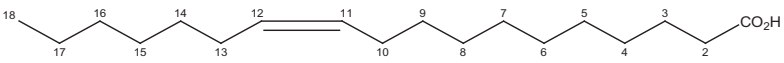
10



11



12



13

Figure 1. Chemical formulae of compounds (**1**–**13**).

214, 273, 335; (MeOH + NaOAc + H_3BO_3) nm: 214, 267, 335; ^{1}H-NMR (500 MHz, CD_3OD) δ: 12.90 (1H, s, 5-OH), 7.96 (1H, s, H-2), 7.27 (2H, dd, *J* = 1.95; 6.84 Hz, H-2′ and H-6′), 6.74 (2H, dd, *J* = 1.95; 6.84 Hz, H-3′ and H-5′), 6.34 (1H, s, H-8), 3.79 (3H, s, OMe-6); ^{13}C-NMR (125 MHz, CD_3OD) δ: 153.77 (d, C-2), 123.08 (s, C-3), 181.44 (s, C-4), 153.92 (s, C-5), 131.76 (s, C-6), 157.62 (s, C-7), 93.90 (d, C-8), 153.40 (s, C-9), 109.99 (s, C-10), 122.15 (s, C-1′), 131.20 (d, C-2′), 115.12 (d, C-3′), 157.95 (s, C-4′), 116.17 (d, C-5′), 130.20 (d, C-6′), 59.74 (q, OCH_3-6). LC-MS (*m*/*z*) 299 [M-H] $^+$, 271 [M-H_2O-H] $^+$.

Irilone (***2***). Yellow powder; UV λ_{max} (MeOH) nm: 205, 265, 330 (sh); (MeOH + NaOMe) nm: 213, 280; (MeOH + $AlCl_3$) nm: 216, 237 (sh), 280, 326 (sh); (MeOH + $AlCl_3$ + HCl) nm: 216, 237 (sh), 282, 363 (sh); (MeOH + NaOAc) nm: 216, 248 (sh), 271, 344 (sh); (MeOH + NaOAc + H_3BO_3) nm: 216, 248 (sh), 271, 344 (sh); ^{1}H-NMR (500 MHz, CD_3OD + $CDCl_3$) δ: 7.82 (1H, s, H-2), 7.30 (2H, brd, *J* = 8.30 Hz, H-2′ and H-6′), 6.82 (2H, brd, *J* = 8.30 Hz, H-3′ and H-5′), 6.44 (1H, s, H-8), 6.02 (2H, brs, OCH_2O); ^{13}C-NMR (125 MHz, CD_3OD + $CDCl_3$) δ: 152.97 (d, C-2), 123.83 (s, C-3), 181.66 (s, C-4), 153.83 (s, C-5), 142.33 (s, C-6), 157.28 (s, C-7), 89.51 (d, C-8), 154.31 (s, C-9), 108.51 (s, C-10), 121.92 (s, C-1′), 130.42 (d, C-2′), 115.62 (d, C-3′), 157.39 (s, C-4′), 115.79 (d, C-5′), 130.30 (d, C-6′), 102.91 (t, OCH_2O). LC-MS (*m*/*z*) 297 [M-H] $^+$, 279 [M-H_2O-H] $^+$.

Nigricin (***3***). Yellow powder; UV λ_{max} (MeOH) nm: 205, 265, 338 (sh); (MeOH + NaOMe) nm: 215, 238 (sh), 282; (MeOH + $AlCl_3$) nm: 209, 262, 321(sh); (MeOH + $AlCl_3$ + HCl) nm: 209, 262, 321 (sh); (MeOH + NaOAc) nm: 213, 265, 327 (sh); (MeOH + NaOAc + H_3BO_3) nm: 213, 265, 327 (sh); ^{1}H-NMR (500 MHz, CD_3OD + $CDCl_3$) δ: 7.73 (1H, s, H-2), 7.27 (2H, dd, *J* = 1.95; 6.35 Hz, H-2′ and H-6′), 6.80 (2H, dd, *J* =1.95; 6.35 Hz, H-3′ and H-5′), 6.57 (1H, s, H-8), 6.00 (2H, brs, OCH_2O), 4.00 (3H, s, OCH_3-5);); ^{13}C-NMR (125 MHz, CD_3OD + $CDCl_3$) δ: 149.65 (d, C-2), 124.67 (s, C-3), 175.27 (s, C-4), 152.15 (s, C-5), 134.34 (s, C-6), 153.98 (s, C-7), 92.19 (d, C-8), 153.03 (s, C-9), 112.56 (s, C-10), 121.99 (s, C-1′), 129.49 (d, C-2′), 114.36 (d, C-3′), 155.99 (s, C-4′), 114.36 (d, C-5′), 129.49 (d, C-6′), 102.40 (t, OCH_2O), 60.01 (q, OCH_3-5). HRESI-MS (*m*/*z*) 312.1140 [M] $^+$ (calculated for $C_{17}H_{12}O_6$, 312.1131).

Iriskumaonin methyl ether (***4***). White powder; UV λ_{max} (MeOH) nm: 205, 266, 321 (sh); (MeOH + NaOMe) nm: 209, 266, 321 (sh); (MeOH + $AlCl_3$) nm: 205, 266, 321 (sh); (MeOH + $AlCl_3$ + HCl) nm: 205, 266, 321 (sh); (MeOH + NaOAc) nm: 214, 266, 321 (sh); (MeOH + NaOAc + H_3BO_3) nm: 214, 266, 321 (sh); ^{1}H-NMR (500 MHz, CD_3OD) δ: 7.73 (1H, s, H-2), 7.12 (1H, d, *J* = 1.96 Hz, H-2′), 6.94 (1H, dd, *J* = 1.96; 8.29 Hz, H-6′), 6.83 (1H, d, *J* = 8.30 Hz, H-5′), 6.57 (1H, s, H-8), 6.00 (2H, brs, OCH_2O), 4.01 (3H, s, OCH_3-5), 3.90 (3H, s, OCH_3-3′), 3.87(3H, s, OCH_3-4′) ; ^{13}C-NMR (125 MHz, CD_3OD) δ: 149.74 (d, C-2), 126.39 (s, C-3), 174.22 (s, C-4), 153.67 (s, C-5), 134.62 (s, C-6), 153.10 (s, C-7), 92.24 (d, C-8), 152.14 (s, C-9), 112.81 (s, C-10), 124.71 (s, C-1′), 111.94 (d, C-2′), 149.41 (d, C-3′), 149.72 (s, C-4′), 110.11 (d, C-5′), 123.66 (d, C-6′), 60.27 (q, OCH_3-5), 55.29 (q, OCH_3-3′), 55.19 (q, OCH_3-4′), 101.21 (t, OCH_2O). HRESI-MS (*m*/*z*) 357.0972 [M] $^+$ (calculated for $C_{19}H_{17}O_7$, 357.0969).

Soforanarin A (***5***). White powder; UV λ_{max} (MeOH) nm: 205, 276; (MeOH + NaOMe) nm: 212, 284; (MeOH + $AlCl_3$) nm: 205, 222 (sh), 284; (MeOH + $AlCl_3$ + HCl) nm: 205, 222 (sh), 284; (MeOH + NaOAc) nm: 213, 217, 274; (MeOH + NaOAc + H_3BO_3) nm: 213, 217, 274; ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 12.70 (1H, s, 5-OH), 7.83 (1H, s, H-2), 7.00 (1H, d, *J* = 2.0 Hz, H-2′), 6.89 (1H, dd, *J* = 2.0;8.29 Hz, H-6′), 6.53 (1H, d, *J* = 8.29 Hz, H-5′), 6.44 (1H, s, H-8), 6.04 (2H, brs, OCH_2O), 3.87 (3H, s, OCH^3-4′). ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 152.65 (d, C-2), 123.38 (s, C-3), 181.22 (s, C-4), 144.23 (s, C-5), 131.57 (s, C-6), 152.85 (s, C-7), 89.41 (d, C-8), 153.54 (s, C-9), 108.12 (s, C-10), 123.60 (s, C-1′), 111.94 (d, C-2′), 145.73 (d, C-3′), 147.16 (s, C-4′), 121.16 (d, C-5′), 114.63 (d, C-6′), 55.19 (q, OCH_3-4′), 101.25 (t, OCH_2O). HRESI-MS (*m*/*z*) 328.0457 [M] $^+$ (calculated for $C_{17}H_{12}O_7$, 328.0463).

Coniferaldehyde (***6***). Yellow powder; UV λ_{max} (MeOH) nm: 222, 242, 335; ^{1}H- (600 MHz, CD_3OD) δ: 9.66 (1H, d, *J* = 7.8 Hz, CHO), 7.42 (1H, d, *J* = 15.5 Hz, H-7), 7.15 (1H, dd, *J* = 1.9; 8.5 Hz, H-5), 7.08 (1H, d, *J* = 1.9 Hz, H-3), 6.97 (1H, d, *J* = 8.5 Hz, H-6), 6.60 (1H, dd, *J* = 7.8; 15.5 Hz, H-8), 3.95 (3H, s, OCH_3-2); ^{13}C-NMR (150 MHz, CD_3OD) δ: 148.92 (s, C-1), 146.72 (s, C-2), 109.28 (d, C-3), 126.73 (s, C-4), 124.00 (d, C-5), 115.17 (d, C-6), 153.26 (d, C-7), 126.44 (d, C-8), 193.57 (d, CHO), 56.03 (q, OCH_3-2). LC-MS (*m*/*z*) 177 [M-H] $^+$.

cis-Epoxyconiferyl alcohol (***7***). Yellowish powder; UV λ_{max} (MeOH) nm: 206, 229, 280; ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 6.83 (1H, d, *J* = 1.95 Hz, H-3), 6.81 (1H, d, *J* = 7.80 Hz, H-6), 6.75 (1H, dd, *J* = 1.95; 7.80 Hz, H-5), 4.67 (1H, d, *J* = 4.39 Hz, H-3′), 4.18 (1H, dd, *J* = 6.83; 9.27; Hz, H-1′*a*), 3.84 (3H, s, OCH_3-2), 3.81 (1H, dd, *J* = 3.42; 9.27 Hz, H-1′*b*), 3.03 (1H, m, H-2′),; ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 144.28 (s, C-1), 145.71 (s, C-2), 107.63 (d, C-3), 131.98 (s, C-4), 117.97 (d, C-5), 113.28 (d, C-6), 70.69 (t, C-1′), 53.20 (d, C-2′), 84.88 (d, C-3′), 54.98 (q, OCH_3-2). HRESI-MS (*m*/*z*) 196.0730 [M] $^+$ (calculated for $C_{10}H_{12}O_4$, 196.0735).

Acetovanillone (***8***). Yellow powder; UV λ_{max} (MeOH) nm: 205, 228, 275, 301; ^{1}H- (500 MHz, CD_3COCD_3) δ: 7.40 (1H, d, *J* = 1.95 Hz, H-2′), 6.78 (1H, d, *J* = 7.81 Hz, H-5′), 7.42 (1H, dd, *J* = 1.95;7.81 Hz, H-6′), 3.80 (3H, s, OCH_3-3′) 2.38 (3H, s, $COCH_3$);); ^{13}C-NMR (125 MHz, CD_3COCD_3) δ: 195.51 (s, C-1), 25.56 (q, C-2), 130.01 (s, C-1′), 110.96 (d, C-2′), 147.70 (s, C-3′), 151.82 (s, C-4′), 114.69 (d, C-5′), 123.62 (d, C-6′), 55.63 (q, OCH_3–3′). HRESI-MS (*m*/*z*) 166.0621[M]$^+$ (calculated for $C_9H_{10}O_3$, 166.0629).

p-Hydroxyacetophenone (***9***). Brown powder; UV λ_{max} (MeOH) nm: 204, 219, 277; ^{1}H-NMR (500 MHz, CD_3COCD_3) δ: 7.74 (2H, d, *J* = 8.30 Hz, H-2′ and H-6′), 6.78 (2H, d, *J* = 8.30 Hz, H-3′ and H-5′), 2.4 (3H, s, $COCH_3$); ^{13}C-NMR (125 MHz, CD_3COCD_3) δ: 195.48 (s, C-1), 26.78 (q, C-2), 129.62 (s, C-1′), 130.82 (d, C-2′ and C-6′), 115.34 (d, C-3′ and C-5′), 162.37 (s, C- 4′). HRESI-MS (*m*/*z*) 136.0530[M]$^+$ (calculated for $C_8H_8O_2$, 136.0524).

3-Hydroxyirisquinone (***10***). Orange powder; UV λ_{max} (MeOH) nm: 203, 287, 422 (sh); ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 5.76 (1H, s, H-5), 5.27 (2H, m, H-10′ and H-11′), 3.78 (3H, s, OCH_3-6), 2.37 (2H, t, *J* = 7.8 Hz, H-1′), 1.94 (4H, m, H-9′ and H-12′), 1.38 (2H, m, H-2′), 1.22 (20H, br s, H-3′- H-8′ and H-13′-H-16′), 0.82 (3H, t, *J* = 7.3 Hz, H-17′); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 181.81 (s, C-1), 118.28 (s, C-2), 150.50 (s, C-3), 180.65 (s, C-4), 101.14 (d, C-5), 160.16 (s, C-6), 21.63 (t, C-1′), 28.19 (t, C-2′), 28.74-28.20 (t, C-3′ and C-7′), 30.89 (t, C-8′), 27.00 (t, C-9′), 128.92 (d, C-10′), 128.89 (d, C-11′), 26.20 (t, C-12′), 30.77 (t, C-13′), 28.76 (t, C-14′), 30.91 (t, C-15′),

21.68 (t, C-16′), 13.07 (q, C-17′), 55.71 (q, OCH_3-6). HRESI-MS (*m*/*z*) 390.9586[M]$^+$ (calculated for $C_{24}H_{38}O_4$, 390.9593).

*7β-Hydroxystigmast-4-en-3-one (**11**)*. White powder; UV λ_{max} (MeOH) nm: 228, 246; ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 5.75 (1H, s, H-4), 4.28 (1H, s, 7-OH), 3.58 (1H, s, H-7α), 1.31 (3H, s, CH_3-19), 0.95 (3H, d, *J* = 6.35 Hz, CH_3-21), 0.86 (3H, t, *J* = 6.83 Hz, CH_3-29), 0.83 (3H, d, *J* = 6.1 Hz, CH_3-26), 0.75 (3H, d, *J* = 6.1 Hz, CH_3-27), 0.68 (3H, s, CH_3-18); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 38.63 (t, C-1), 33.27 (t, C-2), 199.23 (s, C-3), 125.37 (d, C-4), 168.27 (s, C-5), 37.60 (t, C-6), 72.34 (d, C-7), 33.10 (d, C-8), 52.65 (d, C-9), 36.13 (s, C-10), 20.00 (t, C-11), 41.53 (t, C-12), 44.34 (s, C-13), 54.91 (d, C-14), 24.38 (t, C-15), 27.18 (t, C-16), 55.03 (d, C-17), 11.02 (q, C-18), 18.80 (q, C-19), 39.43 (d, C-20), 18.04 (q, C-21), 36.13 (t, C-22), 26.23 (t, C-23), 45.00 (d, C-24), 28.75 (d, C-25), 20.10 (q, C-26), 18.51 (q, C-27), 23.16 (t, C-28), 13.08 (q, C-29). HRESI-MS (*m*/*z*) 428.3642[M] $^+$ (calculated for $C_{29}H_{48}O_2$, 428.3654).

*β-Sitosterol (**12**)*. White powder; UV λ_{max} (MeOH) nm: 203; ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 5.29 (1H, m, H-6), 3.48 (1H, m, H-3α), 0.96 (3H, s, CH_3-19), 0.93 (3H, d, *J* = 6.43 Hz, CH_3-21), 0.84 (3H, d, *J* = 6.84 Hz, CH_3-26), 0.80 (3H, d, *J* = 6.84 Hz, CH_3-27), 0.78'de (3H, t, *J* = 7.82 Hz, CH_3-29), 0.61 (3H, s, CH_3-18).

*cis-Vaccenic acid (**13**)*. Colourless oil; UV λ_{max} (MeOH) nm: 203; ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 5.2–5.3 (2H, m, H-11 and H-12), 2.28 (2H, t, *J* = 7.33 Hz, H-2), 1.94 (4H, m, H-10 and H-13), 1.58 (2H, pentet, *J* = 7.83 Hz, H-17), 1.12–1.30 (20H, m, H-3 - H-9 and H-14 - H-16), 0.81 (3H, t, *J* = 7.32 Hz, CH_3-18); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 32.83 (t, C-2), 129.03 (d, C-11), 128.73 (d, C-12), 30.92-21.68 (t, 13 × CH_2), 13.09 (q, C-18), 179.82 (s, COOH). HRESI-MS (*m*/*z*) 282.2550 [M]$^+$ (calculated for $C_{18}H_{34}O_2$, 282.2558).

Determination of total phenolic content. The concentrations of phenolic content in all crude extracts were expressed as micrograms of pyrocatechol equivalents (PEs), determined with FCR according to the method of Slinkard and Singleton (1977). The concentration of phenolic compounds was calculated according to the following equation that was obtained from standard pyrocatechol graphic:

$$\text{Absorbance} = 0.0321\ \text{pyrocatechol}\ (\mu g) - 0.0457 \quad (R^2 = 0.9902)$$

Determination of total flavonoid content. Measurement of flavonoid content of the crude extracts was based on the method described by Moreno *et al*. (2000) with a slight modification and results were expressed as quercetin equivalents. The concentration of flavonoid compounds was calculated according to the following equation:

$$\text{Absorbance} = 0.0751\ \text{quercetin}\ (\mu g) - 0.0652 \quad (R^2 = 0.9985)$$

Determination of the antioxidant activity with the β-carotene bleaching method. The antioxidant activity of the samples was evaluated using the β-carotene–linoleic acid test system (Miller, 1971).

Cupric reducing antioxidant capacity (CUPRAC). Cupric reducing antioxidant capacity of the samples was determined according to the method described by Apak *et al*. (2004).

Anticholinesterase activity. Acetyl- (AChE) and butyryl-cholinesterase (BChE) inhibitory activities of the samples were measured by slightly modifying the spectrophotometric method developed by Ellman *et al*. (1961).

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

RESULTS AND DISCUSSION

The total phenolic and flavonoid contents of the crude extracts prepared from the rhizomes of *I. suaveolens* were determined as pyrocatechol and quercetin equivalents, respectively (Table 1). The chloroform extract was the richest extract in phenolic compounds (437.69 ± 0.82 µg), and the methanol extract in flavonoid compounds (528.15 ± 0.68 µg) among the tested extracts. Figure 2A shows the total antioxidant activity of the crude extracts, compared with quercetin and BHT, determined by the β-carotene bleaching method. The total antioxidant activity increased with increasing amounts of the extracts. None of the extracts showed greater antioxidant activity than the standard compounds. The petroleum ether and chloroform extracts, having almost the same inhibition of lipid peroxidation at 80 µg/mL, indicated higher inhibition than the methanol extract at all concentrations (Fig. 2A).

Figure 3A shows the cupric reducing power of the crude extracts and the standard compounds, quercetin and BHT, using the CUPRAC method. The cupric reducing antioxidant capacity increased with increasing amounts of the extracts. The chloroform extract exhibited almost the same reducing power with the standards at 100 µg/mL, and also indicated stronger activity than the tested extracts at all concentrations.

The anticholinesterase activity of the crude extracts was compared with galantamine at 200 µg/mL (Table 2). The petroleum ether extract exhibited moderate anticholinesterase activity. The chloroform extract showed significantly inhibition against BChE (75.03 ± 1.29%), while it was found to be inactive against AChE. Since

Table 1. Total phenolic and flavonoid contents of the extracts[a]

Extract	Phenolic content (µg PEs/mg extract)[b]	Flavonoid content (µg QEs/mg extract)[c]
ISUP	139.04 ± 0.50	57.11 ± 0.45
ISUC	437.69 ± 0.82	246.82 ± 0.56
ISUM	266.40 ± 1.00	528.15 ± 0.68

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

[b] PEs, pyrocatechol equivalents.

[c] QEs, quercetin equivalents.

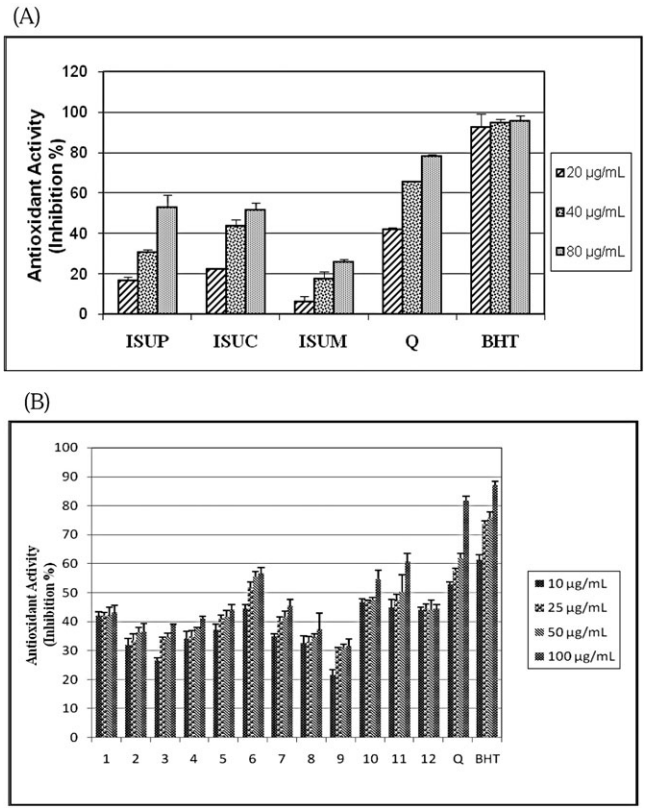


Figure 2. Inhibition (%) of lipid peroxidation of (A) the extracts, (B) the compounds (**1–12**), quercetin and BHT by β-carotene bleaching method. Values are mean ± SD, $n = 3$, $p < 0.05$, significantly different with Student's *t*-test.

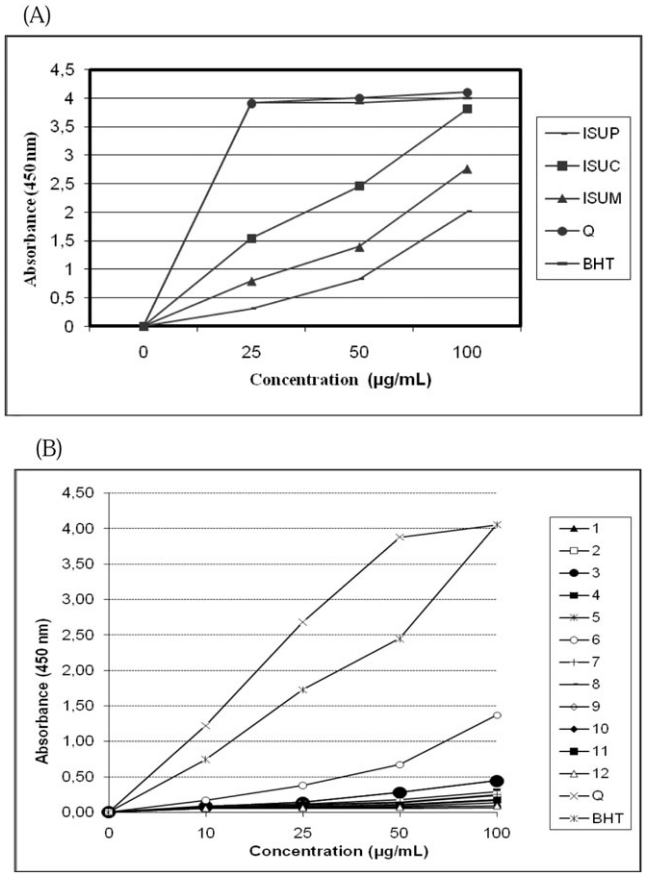


Figure 3. Cupric reducing antioxidant capacity of (A) the extracts, (B) the compounds (**1–12**), quercetin and BHT. Values are mean ± SD, $n = 3$, $p < 0.05$, significantly different with Student's *t*-test.

the petroleum ether and chloroform extracts possessed higher antioxidant and anticholinesterase effects than the methanol extract, they were separated using chromatographic methods to isolate their bioactive constituents.

Flavonoids are widespread metabolites in the family Iridaceae with chemotaxonomic significance (Boltenkov *et al*., 2005). *Iris* species are known to be rich in isoflavonoids and flavonoids, in addition, quinones and phenols in some taxa (Farag *et al*., 1999; Marner and Horper, 1992). The first phytochemical investigation of *I. suaveolens* Boiss. & Reuter resulted in the isolation of 13 known compounds, five of which were isoflavones (**1–5**), four phenolics (**6–9**), a quinone (**10**), two steroids (**11, 12**) and a fatty acid (**13**). The compounds (**1–7, 9**) were obtained from the chloroform extract and the others (**8, 10–13**) from the petroleum ether extract. Isoflavones (**1–5**), phenolics (**6–9**) and a quinone (**10**) of *I. suaveolens* found in this study are in agreement with the results of the previous phytochemical studies performed on other *Iris* species. The structural determination of the isolates (**1–13**) was established using spectral methods, and their spectroscopic data were in full agreement with those of standard samples.

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). In this study, the antioxidant capacity of the crude extracts and the isolated compounds (**1–4**, **6–12**) were compared with their anticholinesterase activity. Coniferaldehyde

Table 2. Anticholinesterase activity of the extracts at 200 µg/mL[a]

Extract	Inhibition % against AChE	Inhibition % against BChE
ISUP	40.58 ± 0.75	57.24 ± 0.71
ISUC	na	75.03 ± 1.29
ISUM	na	25.91 ± 0.29
Galantamine[b]	89.99 ± 0.11	87.17 ± 0.83

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

[b] Standard drug.

Na, not active at 200 µg/mL.

(**6**) having the highest activity in the β-carotene bleaching method at 25 and 50 µg/mL, and in the CUPRAC assay at 10, 25, 50 and 100 µg/mL exhibited almost the same butyrylcholinesterase inhibitory activity (82.60 ± 2.33%) as galantamine, a positive control (Fig. 2B-3B, Table 3). Although 7β-hydroxystigmast-4-en-3-one (**11**) showed the highest inhibition of lipid peroxidation by the β-carotene bleaching method at 100 µg/mL, it was found to be inactive against acetyl- and butyrylcholinesterases (Fig. 2B, Table 3). 3-Hydroxyirisquinone (**10**), having moderate inhibition of lipid peroxidation, exhibited the best anticholinesterase activity among the tested compounds (Fig. 2B, Table 3). Irilone (**2**) and nigricin (**3**) possessing almost the same inhibition of lipid peroxidation showed significant inhibition against

Table 3. Anticholinesterase activity of compounds (1–4, 6–12) at 200 μg/mL[a]

Compound	Inhibition % against AChE	Inhibition % against BChE
1	na	47.34 ± 0.91
2	na	71.34 ± 0.13
3	na	80.13 ± 0.13
4	13.64 ± 0.61	35.07 ± 0.39
6	20.32 ± 0.65	82.60 ± 2.33
7	na	75.55 ± 0.39
8	na	30.16 ± 0.33
9	30.01 ± 0.91	42.95 ± 2.72
10	57.07 ± 0.16	69.69 ± 0.91
11	na	20.79 ± 1.12
12	na	12.45 ± 1.59
Galantamine[b]	76.06 ± 0.51	86.26 ± 0.66

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

[b] Standard drug.

Na, not active at 200 μg/mL.

butyrylcholinesterase, 71.34% and 80.13%, respectively (Fig. 2B, Table 3). *cis*-Epoxyconiferyl alcohol (**7**) demonstrated also significant butyrylcholinesterase inhibitory activity (75.55 ± 0.39%). Among the tested compounds, only coniferaldehyde exhibited a weak activity in the CUPRAC assay which is one of the electron transfer-based methods.

In conclusion, coniferaldehyde (**6**), *cis*-epoxyconiferyl alcohol (**7**), *p*-hydroxyacetophenone (**9**), 7β-hydroxystigmast-4-ene-3-on (**11**) and *cis*-vaccenic acid (**13**) were not reported earlier from the genus *Iris*. The antioxidant activity of the isolates (**3–6, 9–11**) and the anticholinesterase activity of the compounds (**1–4**, **6–12**) were also determined for the first time in this study.

Most of the isolates (**1–10**) belong to the phenolic class of compounds. The lipid peroxidation inhibition and anticholinesterase activities of the petroleum ether and chloroform extracts were related to the presence of these phenolic compounds. This study proved also that *Iris* species may be potentially a source of natural antioxidants and anticholinesterases, and they could be used in pharmaceutical, cosmetic and perfume industries.

Acknowledgements

This work was supported by Research Fund of the Istanbul University (Project number: 2907). We thank Professor Dr Franz-Joseph Marner for providing laboratory facilities (Department of Chemistry, Institute of Biochemistry, University of Cologne, Cologne, Germany).

Conflict of Interest

The authors have declared that there is no conflict of interest.

REFERENCES

Al-Khalil S, Al-Eisawi D, Kato M, Iinuma M. 1994. New isoflavones from *Iris nigricans*. *J Nat Prod* **57**: 201–205.

Apak R, Guclu K, Ozyurek M, Karademir SE. 2004. Novel total antioxidant capacity index for dietary polyphenols and vitamins C and E, using their cupric ion reducing capability in the presence of neocuproine: CUPRAC method. *J Agric Food Chem* **52**: 7970–7981.

Atta-ur-Rahman, Nasim S, Baig I *et al*. 2003. Anti-inflammatory isoflavonoids from the rhizomes of *Iris germanica*. *J Ethnopharmacol* **86**: 177–180.

Atta-ur-Rahman, Nasim S, Baig I *et al*. 2004. Two new isoflavonoids from the rhizomes of *Iris soforana*. *Nat Prod Res* **18**: 465–471.

Baytop T. 1984. *Therapy with Medicinal Plants in Turkey*. Istanbul University Press: Istanbul.

Boltenkov EV, Rybin VG, Zarembo EV. 2005. Flavones from callus tissue of *Iris ensata*. *Chem Nat Comp* **41**: 539–541.

Carcache-Blanco EJ, Cuendet M, Park EJ *et al*. 2006. Potential cancer chemopreventive agents from *Arbutus unedo*. *Nat Prod Res* **20**: 327–334.

Crestini C, D'Auria M. 1997. Singlet oxygen in the photodegradation of lignin models. *Tetrahedron* **53**: 7877–7888.

Duffy PE, Quinn SM, Roche HM, Evans P. 2006. Synthesis of *trans*-vaccenic acid and *cis*-9-*trans*-11-conjugated linoleic acid. *Tetrahedron* **62**: 4838–4843.

Ellman GL, Courtney KD, Andres V, Featherstone RM. 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem Pharmacol* **7**: 88–95.

Farag SF, Backheet EY, El-Emary NA, Niwa M. 1999. Isoflavonoids and flavone glycosides from the rhizomes of *Iris carthaliniae*. *Phytochemistry* **50**: 1407–1410.

Farag SF, Kimura Y, Ito H, Takayusu J, Tokuda H, Hatano T. 2009. New isoflavone glycosides from *Iris spuria* L. (Calizona) cultivated in Egypt. *J Nat Med* **63**: 91–95.

Gu LW, Weng XC. 2001. Antioxidant activity and components of *Salvia plebeia*. *Food Chem* **73**: 299–305.

Guz NR, Stermitz FR. 2000. Spectral comparisons of coniferyl and cinnamyl alcohol epoxide derivatives with a purported *cis*-epoxyconiferyl alcohol isolate. *Phytochemistry* **54**: 897–899.

Hanawa F, Tahara S, Mizutani J. 1991. Isoflavonoids produced by *Iris pseudacorus* leaves treated with cupric chloride. *Phytochemistry* **30**: 157–163.

Howes MJR, Perry NSL, Houghton PJ. 2003. Plants with traditional uses and activities relevant to the management of Alzheimer's disease and other cognitive disorders. *Phytother Res* **17**: 1–18.

Kolak U, Topçu G, Birteksöz S, Ötük G, Ulubelen A. 2005. Terpenoids and steroids from the roots of *Salvia blepharochlaena*. *Turk J Chem* **29**: 177–186.

Marner FJ, Horper W. 1992. Phenols and quinones from seeds of different *Iris* species. *Helv Chim Acta* **75**: 1557–1562.

Marner FJ, Krick W, Gellrich B, Jaenicke L. 1982. Irigermanal and iridogermanal: Two new triterpenoids from rhizomes of *Iris germanica* L. *J Org Chem* **47**: 2531–2536.

Marner FJ, Simic K, Scholz B, Küster B. 1995. Isolation and structure determination of a new iridal from *Iris sibirica*. *J Nat Prod* **58**: 299–301.

Mathew B. 1984. *Iris* L. In *Flora of Turkey and East Aegean Islands*, Davis PH (ed.). Vol. 8. University Press: Edinburgh, 381–382, 395–396.

Miller HE. 1971. A simplified method for the evaluation of antioxidants. *J Am Oil Chem Soc* **48**: 91.

Moreno MIN, Isla MI, Sampietro AR, Vattuone MA. 2000. Comparison of the free radical-scavenging activity of propolis from several regions of Argentina. *J Ethnopharmacol* **71**: 109–114.

Seki K, Haga K, Kaneko R. 1995. Phenols and a dioxotetrahydrodibenzofuran from seeds of *Iris pallasii*. *Phytochemistry* **38**: 965–973.

Senatore F, Nunziata A, D'Agostino M, De Feo V. 1999. Flavonol glycosides and *p*-hydroxyacetophenone from *Chuquiraga spinosa*. *Pharm Biol* **37**: 366–368.

Slinkard K, Singleton VL. 1977. Total phenol analyses: Automation and comparison with manual methods. *Am J Enol Viticult* **28**: 49–55.

Sy LK, Brown GD. 1999. Coniferaldehyde derivatives from tissue culture of *Artemisia annua* and *Tanacetum parthenium*. *Phytochemistry* **50**: 781–785.

Veitch NC, Sutton PSE, Kite GC, Ireland HE. 2003. Six new isoflavones and a 5-deoxyflavonol glycoside from the leaves of *Ateleia herbert-smithii*. *J Nat Prod* **66**: 210–216.