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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

A new isoflavone from *Blepharis ciliaris* of an Egyptian origin

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Abstract A phytochemical study of the aerial parts of *Blepharis ciliaris* (L.) B.L. Burtt. led to the isolation of one new isoflavone glycoside caffeic acid ester: genistein-7-*O*-(6″-*O*-E-caffeoyl-*β*-D-glucopyranoside) (**4**), along with seven known compounds: methyl veratrate (**1**), methyl vanillate (**2**), protocatechuic acid (**3**), naringenin-7-*O*-(3″-acetyl-6″-E-*p*-coumaroyl-*β*-D-glucopyranoside) (**5**), naringenin-7-*O*-(6″-E-*p*-coumaroyl-*β*-D-glucopyranoside) (**6**), apigenin-7-*O*-(6″-E-*p*-coumaroyl-*β*-D-glucopyranoside) (**7**), and acteoside (**8**). Their structures were established on the basis of detailed analyses of physical, chemical, and spectral data. Compounds **1**, **2**, **3**, **6**, and **8** were isolated for the first time from this plant. The antioxidant activity of the different extracts as well as for some of the isolated compounds was evaluated.

Keywords *Blepharis ciliaris* · Isoflavone · Naringenin · Apigenin · Acteoside · Antioxidant

Introduction

The family Acanthaceae includes about 346 genera and around 4,300 species, distributed in tropics, the Mediterranean, Australia, Central America, Brazil, Africa, and Indo-Malaysia (Ghazanfar, 1994; Lawrence, 1968; Meikle, 1985). Several plants of the family Acanthaceae are medicinally useful and others are economically important as ornamentals since they have large flowers with colorful petals and could be considered as a source of natural dyes (Chopra, 1973). The genus *Blepharis* is represented in Egypt (Sinai peninsula) and Saudi Arabia only by *Blepharis ciliaris* (L.) B.L. Burtt. which is known in arabic as Shawk-ul-Dab, Zughaf, Shuqaf (Boulos, 1981; Täckholm, 1974; Muschler, 1970; Rizk, 1986). The plant seeds (roasted or crushed) are applied on sores, wounds, and boils as an antibacterial. The seeds are also considered as diuretic, aphrodisiac, and expectorant (Deshpande, 2006). The charcoal from the roots is applied to the eyes to improve vision, hence the Arabic name "Kohl-el-agouz" (Boulos, 1981; Täckholm, 1974). Previous phytochemical studies of *B. ciliaris* (L.) resulted in the isolation and identification of apigenin 7-*O*-(3″-acetyl-6″-E-*p*-coumaroylglucoside), naringenin 7-*O*-(3″-acetyl-6″-E-*p*-coumaroylglucoside), apigenin-7-*O*-glucoside, and 9′-decarboxy-rosmarinic acid-4′-*O*-(1 → 4)-galactosylrhamnoside (Afifi, 2003; Harraz *et al.*, 1996).

Materials and methods

General

Melting points were uncorrected and carried out on an Electrothermal 9100 Digital Melting Point apparatus (Electrothermal Engineering Ltd, Essex, England). EI- and FAB–MS were recorded on a Jeol the mass route JMS.600 H mass spectrometer. UV spectra were recorded in MeOH on a Shimadzu 1601 UV/VIS spectrophotometer. The IR spectra were measured on a Shimadzu Infrared-400 spectrophotometer (Kyoto, Japan). NMR spectra (chemical shifts in ppm, coupling constants in Hz) were recorded on

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Varian Oxford NMRYH-400 using DMSO-d6 as solvent. NMR spectra were referenced to the solvent signal (2.49 ppm for 1H and 39.9 ppm for 13C). Column chromatographic separation was performed on silica gel 60 (0.04–0.063 mm), RP-18 (0.04–0.063 mm, Merck), and Sephadex LH-20 (0.25–0.1 mm, Merck). TLC was performed on precoated TLC plates with silica gel 60 F254 (0.2 mm, Merck). The solvent systems were used for TLC analyses; $CHCl_3$:MeOH (9.5:0.5, I), (9:1, II), (8:2, III), (7:3, IV), and *n*-BuOH:acetone:formic acid:H_2O 60:17:8:15 (system V). Authentic flavonoids and sugars were obtained from the Department of Pharmacognosy, Faculty of Pharmacy, Assiut University. 1,1-Diphenyl-2-picrylhydrazyl radical (DPPH) and quercetin were purchased from Sigma Chemical Co. (Germany).

Plant materials

Blepharis ciliaris (L.) B.L. Burtt. aerial parts were collected on May 2005 from Southern Sinai (Wadi Al-Ratam). The plant was kindly identified by Dr. A. A. Fayed, Prof. of Plant Taxonomy, Faculty of Science, Assiut University, Assiut, Egypt. A voucher sample (BC-20051) was kept in the herbarium of the Faculty of Pharmacy, Assiut University, Assiut, Egypt.

Chemistry

Extraction and isolation

The air-dried powdered aerial parts (3 kg) were exhaustively extracted by cold percolation with MeOH. The alcoholic extract was concentrated under reduced pressure to afford a viscous brown residue (250 g). The residue was mixed with 500 mL distilled H_2O and subjected to solvent fractionation using *n*-hexane, $CHCl_3$, EtOAc, and *n*-BuOH which were separately concentrated yielding 70, 40, 40, and 50 g, respectively. The $CHCl_3$ fraction (40 g) was subjected to silica gel column chromatography using $CHCl_3$:MeOH gradient elution. Ten subfractions were obtained (CB-I to CB-X). All fractions were monitored by TLC and sprayed with $FeCl_3$ and 10 % v/v H_2SO_4, and then heated to 110 °C. The subfraction (CB-II) (2 g) was subjected to silica gel CC (60 g, 100 × 4 cm) using $CHCl_3$:MeOH gradients, to afford **1** (25 mg, colorless needles, $CHCl_3$:MeOH 9.5:0.5) and **2** (20 mg, colorless needles, $CHCl_3$:MeOH 9.5:0.5). While the subfraction CB-III (1 g) was subjected to silica gel CC (30 g, 100 × 1 cm) using $CHCl_3$:MeOH gradient elution where compound **3** (20 mg, pale yellow needles, $CHCl_3$:MeOH 9:1) was isolated; the EtOAc fraction (40 g) was subjected to silica gel CC using $CHCl_3$:MeOH gradient elution. Twelve subfractions were obtained (EB-I to EB-XII). All subfractions were monitored by TLC and sprayed by 5 % AlCl3 and 10 % v/v H_2SO_4, and then heated to 110 °C. Flavonoidal subfractions (EB-II to EB-V) were subjected to silica gel CC (80 g, 100 × 4 cm) using $CHCl_3$: MeOH gradients, compounds **4** (200 mg, yellow needles, $CHCl_3$:MeOH 9:1), **5** (25 mg, yellow powder, $CHCl_3$:MeOH 8:2), **6** (100 mg, yellow powder, $CHCl_3$:MeOH 8:2), and **7** (40 mg, pale yellow powder, $CHCl_3$:MeOH 8:2) were isolated. Reversed phase CC of subfraction (EB-VI, 80 mg) (30 g, 60 × 3 cm) using H_2O:MeOH gradient elution gave compound **8** (30 mg, dark red residue, H_2O:MeOH 6:4). The other fractions were kept for further investigation.

Genstein-7-*O*-(6″-*O*-E-caffeoyl)-*β*-D-glucopyranoside (**4**): Yellow needles (MeOH). Rf = 0.75 (solvent system II). m.p 234 °C. UV λ_{max} (MeOH) nm: 270, 301. IR (KBr): γ_{max} 3270, 1690, 1605, 1509 cm^{-1}. NMR data (DMSO-d_6, 400 and 100 MHz): see Table 1 FABMS *m/z*: 595 $[M+H]^+$, 433 [M-(caffeoyl unit)+H]$^+$, 271 [M-(caffeoyl-unit+hexose unit)+H]$^+$.

Antioxidant activity

The antioxidant activity was determined as previously outlined (Steffan *et al*., 2005) by the decrease in the absorption of each of the isolated compounds or soluble fractions in 118 × 10^{-5} % DPPH solution (final concentration of the sample in the cuvette was 20 μM for pure compounds and 0.25, 0.5, and 1.0 mg/mL for soluble fractions) monitored at 517 nm using a spectrophotometer. The absorbance of DPPH in EtOH (with or without compounds) was measured after 2 min. The antioxidant activity of each compound was measured in relation to quercetin (as a reference antioxidant) set as 100 % antioxidant activity. Determinations were performed in triplicate. The antioxidant activity was calculated using the following equation:$\%\ \text{Antioxidant activity} = 1 - \frac{A^\circ \text{ with compound}}{A^\circ \text{ of the blank}} \times 100$ where, A° = Absorbance

Acid hydrolysis

A solution of the isolated glycoside (5 mg in 10 mL MeOH) was treated with 3 % H_2SO4 (1.5 mL) and heated at 100 °C for 1 h. The aglycone was extracted with EtOAc, concentrated under reduced pressure, purified on Sephadex LH-20 column using MeOH, and identified by co-TLC with an authentic sample using solvent system II. The sugars in the aqueous layer were identified by co-PC (paper chromatography) with authentic materials using solvent system V.

Results and discussion

In this paper, we report the isolation and structural elucidation of one new isoflavone glucoside [genistein-7-*O*-(6″-*O*-E-caffeoyl-*β*-D-glucopyranoside)] (**4**), along with seven

Table 1 ^{1}H and ^{13}C-NMR data of **4** (DMSO-d6, 400 MHz)

No.	δ_C (mult.)	δ_H [mult., J (Hz)]
2	156.9 (CH)	8.43 s
3	121.7 (C)	–
4	181.9 (C)	–
5	162.2 (C)	–
6	99.4 (CH)	6.45 brs
7	164.4 (C)	–
8	94.6 (CH)	6.55 brs
9	161.1 (C)	–
10	105.3 (C)	–
1′	120.3 (C)	–
2′, 6′	130.1 (CH)	7.33 d (8.8)
3′, 5′	115.8 (CH)	6.67 d (7.2)
4′	160.2 (C)	–
1″	102.8 (CH)	5.17 d (8.0)
2″	73.0 (CH)	3.14–5.03
3″	73.7 (CH)	
4″	69.9 (CH)	
5″	76.3 (CH)	
6″	63.4 (CH_2)	
1‴	124.6 (C)	–
2‴	115.98 (CH)	6.81 d (3.2)
3‴	145.1 (C)	–
4‴	147.3 (C)	–
5‴	116.1 (CH)	7.90 d (8.0)
6‴	120.3 d	6.93 dd (8.0, 3.2)
7‴	145.0 d	7.45 d (15.6)
8‴	113.4 d	6.30 d (15.6)
9‴	166.6 (C)	–
5-OH	–	12.92

known compounds: methyl veratrate (**1**), methyl vanillate (**2**), protocatechuic acid (**3**), naringenin-7-*O*-(3″-acetyl-6″-E-*p*-coumaroyl-*β*-D-glucopyranoside) (**5**), naringenin-7-*O*-(6″-E-*p*-coumaroyl-*β*-D-glucopyranoside) (**6**), apigenin-7-*O*-(6″-E-*p*-coumaroyl-*β*-D-glucopyranoside) (**7**), and acteoside (**8**) from the aerial parts of *B. ciliaris*. Compounds **1**–**3**, **6**, and **8** are reported for the first time from this plant (Fig. 1). The antioxidant activity of the crude extracts, and two of the isolated compounds were evaluated.

Compound **4** was isolated as yellow needles and gave positive tests for flavonoids (Harborne and Mabry, 1975; Mabry *et al*., 1970; Markham, 1982). The FABMS spectrum showed $[M+H]^+$ at *m/z* 595 consistent with molecular formula $C_{30}H_{27}O_{13}$. The two significant fragments at *m/z* 433 $[M\text{-caffeoyl}+H]^+$ and *m/z* 271 $[M\text{-caffeoyl,-hexose,}+H]^+$ indicated that **4** is a favonoid with caffeoyl and hexose units. The characteristic fragments in the mass spectrum suggested it to be a genistein derivative (Mabry *et al*., 1970). The UV spectrum in MeOH displayed absorption band at λ_{max} 270 and 301 nm which are characteristic for isoflavonoid (Mabry *et al*., 1970). The bathochromic shift of the UV absorption bands I and II with $NaOCH_3$ and $AlCl_3$ suggested the presence of 4′- and 5- OH groups, respectively, and the absence of NaOAc band II shift indicated that the 7-OH group was substituted (Mabry *et al*., 1970). The IR spectrum indicated the presence of an *α*,*β*-unsaturated CO group at 1690 cm^{-1}, OH groups at 3270 cm^{-1}, and aromatic ring at 1605 and 1509 cm^{-1} (Silverstein and Webster, 1998). The ^{1}HNMR spectrum of **4** showed two singlet signals at δ_H 12.92 and 8.43 which were assigned to 5-chelated OH group and H-2 of isoflavone (Table 1) (Agrawal, 1989; Harborne, 1988). The protons resonances at δ_H 7.33 (2H, d, $J = 8.8$ Hz) and δ_H 6.67 (2H, d, $J = 8.8$ Hz) indicated the presence of a para-disubstituted benzene moiety (ring B) as well as other resonated protons at δ_H 6.55 and 6.45 (each 1H, brs) which were assigned to H-8 and H-6, respectively (Agrawal, 1989; Harborne, 1988; Williams and Harborne, 1994). The ^{1}HNMR spectrum of **4** also displayed an ABX system for a 1,3,4-tri-substituted aromatic ring at δ_H [6.81 (1H, d, $J = 3.2$ Hz, H-2‴), 7.90 (1H, d, $J = 8.0$ Hz, H-5‴), 6.93 (1H, dd, $J = 8.0$, 3.2 Hz, H-6‴)], and two trans-olefinic protons at δ_H [7.45 (1H, d, $J = 15.6$ Hz, H-7‴), 6.30 (1H, d, $J = 15.6$ Hz, H-8‴)], suggesting the presence of caffeoyl moiety (Chen and Yang, 2007; Dürüst *et al*., 2001). Furthermore, anomeric proton for sugar moiety at δ_H 5.17 (1H, d, $J = 8.0$ Hz) indicated *β*-glucopyranose moiety (Agrawal, 1992). The presence of genistein, *β*-D-glucose, and caffeoyl moiety were confirmed by ^{13}CNMR (Table 1) and the multiplicity of each carbon was determined by DEPT experiments. Upon the hydrolysis of **4**, genistein, caffeic acid, and *β*-D-glucose were identified by co-TLC alongside with authentic samples. On the basis of the above results, it was assumed that **4** contained caffeoyl glucose linked at the 7-OH group of genistein. Furthermore, the significant downfield shift of C-6″ of glucose (δ_C 63.4) indicated the attachment of caffeoyl moiety at C-6″ of glucose. On the basis of the previous mentioned data, **4** was identified as genistein-7-*O*-(6″-*O*-*E*-caffeoyl)-*β*-D-glucopyranoside and considered as a new natural product.

The other isolated compounds were identified as methyl veratrate (**1**) (Erickson and Miksche, 1974), methyl vanillate (**2**) (Wu *et al*., 1994), protocatechuic acid (**3**) (Gerothanassis *et al*., 1998; Sang *et al*., 2002), naringenin-7-*O*-(3″-acetyl-6″-E-*p*-coumaroyl-*β*-D-glucopyranoside) (**5**) (Harraz *et al*., 1996), naringenin-7-*O*-(6″-E-*p*-coumaroyl-*β*-D-glucopyranoside) (**6**) (Singh *et al*., 1999), apigenin-7-*O*-(6″-E-*p*-coumaroyl-*β*-D-glucopyranoside) (**7**) (Harraz *et al*., 1996), and acteoside (**8**) (Pereira *et al*., 2008) by comparison of their physical and spectral data with those in literature.

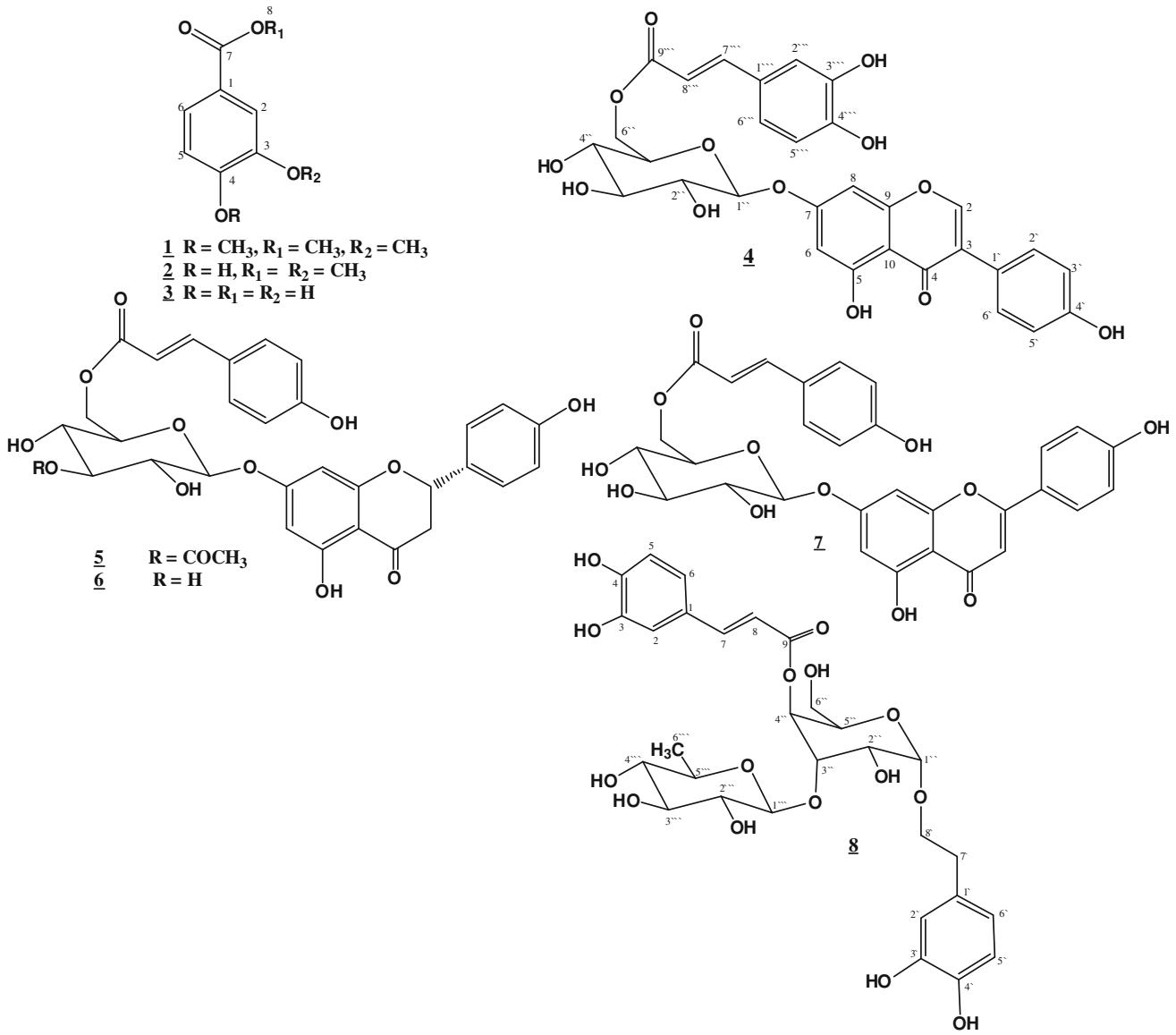

Fig. 1 Chemical structures of the isolated compounds

The results of antioxidant activity proved that the total MeOH, EtOAc, and aqueous extracts of *B. ciliaris* exhibited high antioxidant activities of 89, 86, and 85 %, respectively, while compounds **4** and **6** showed no activity. The activity of the different extracts may be due to the presence of phenolic compounds (flavonoids, phenylpropanoids, and phenolic acids).

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