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# Anticholinesterase furocoumarins from *Heracleum platytaenium*, a species endemic to the Ida Mountains

**Demet DİNCEL,[1,2] Seda Damla HATİPOĞLU,[1,3] Ahmet Ceyhan GÖREN,[3] Gülaçtı TOPÇU[1,2,*]**

[1]Department of Chemistry, Faculty of Science and Letters, İstanbul Technical University, İstanbul, Turkey
[2]Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Bezmialem Vakıf University, İstanbul, Turkey
[3]TÜBİTAK National Metrology Institute (UME), Chemistry Group Laboratories, Gebze, Kocaeli, Turkey

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**Abstract:** The petroleum ether extract of *Heracleum platytaenium* afforded 8 furocoumarins (psoralen, bergapten, xanthotoxin, pimpinellin, isopimpinellin, sphondin, byakangelicin, and heraclenol) and the methanol extract of *H. platytaenium* gave only 1 glycosylated dihydrofurocoumarin, apterin. In addition, stigmasterol was also obtained from petroleum ether extract. Structure identification of the isolated compounds has been achieved by using spectroscopic methods, namely 1D and 2D NMR experiments and mass spectral analyses. The antioxidant activity of the extracts and pure compounds was investigated by 2 methods, including DPPH free radical scavenging activity and lipid peroxidation inhibitory activity by $\beta$-carotene-linoleic acid assays. The anticholinesterase activity of petroleum ether and methanol extracts of the plant and the isolated furocoumarins was investigated against acetylcholinesterase and butyrylcholinesterase enzymes by the Ellman method in vitro.

**Key words:** *Heracleum platytaenium*, furocoumarin, antioxidant activity, anticholinesterase activity, pimpinellin, sphondin

## 1. Introduction

The genus *Heracleum* is one of the largest genera of the family Apiaceae, with 125 species spread throughout the world. The genus is widely distributed in Asia with 109 species and is represented by 23 species in Turkey, 9 of which are endemic.[1] *Heracleum* species are generally known as "baldırgan otu" or "tavşancık otu" in Anatolia. Tea is made from the leaves and fruits of *H. platytaenium* and it is used as folkloric drug for gastritis, for enteritis, and in the treatment of epilepsy.[2] Several *Heracleum* species have been used traditionally for many purposes in different countries. The roots of some species of *Heracleum* are used in folk medicine as analgesic,[3] antipyretic,[4] antiseptic, and carminative. The *Heracleum* species are also used as spices[5] and flavoring agents traditionally.[6] The essential oil of *H. persicum* has analgesic and antiinflammatory effects.[7] The extracts of *H. maximum* Bartr. roots were investigated for antibiotic activity against *Mycobacterium tuberculosis* and *Mycobacterium avium*.[8] The acetone extract of the seeds of *H. persicum* showed a dose-dependent anticonvulsant activity.[9]

The compounds typically found in this genus are coumarins and generally furocoumarins, furocoumarin dimers,[10] coumarin glycosides,[11] anthraquinones and stilbene derivatives,[12] and flavonoids.[13] Coumarins

*Correspondence: gtopcu@bezmialem.edu.tr
Dedicated to the memory of Prof Dr Ayhan Sıtkı Demir for his pioneering studies to the organic chemistry.

include a large group of natural products widely distributed in the plant kingdom, especially in the families of Apiaceae, Rutaceae, Fabaceae, and Asteraceae. They have many biological effects and pharmacological activities.[14,15] *H. platytaenium* extracts and essential oil exhibited antimicrobial and anticandidal activities.[16,17] Coumarins from *H. candicans* Wall were reported to possess nematicidal activity.[18] Furocoumarins from *H. persicum*, *H. sibiricum*, and *H. verticillatum* and furocoumarins and essential oil from the fruits of *H. crenatifolium* were reported to have anticonvulsant activity.[19]

This is the first phytochemical report on the isolation and structure elucidation of 8 known furocoumarins and a known steroid stigmasterol from petroleum ether extract, and a dihydrofurocoumarin glycoside apterin from the methanol extract of *H. platytaenium*, with antioxidant and anticholinesterase activities, which is an endemic species to the Ida Mountains (Kazdağları) of Turkey.

## 2. Results and discussion

Coumarin mixture was obtained from the petroleum ether of the aerial parts of *H. platytaenium*. The exhausted plant was subsequently macerated with methanol. Purification of 8 furocoumarins from the petroleum ether extract and a dihydrofurocoumarin glycoside from the methanol extract was carried out by repeated preparative thin layer chromatography (TLC). Their structures were identified by NMR ($^1$H, APT, HMBC, and HSQC) and

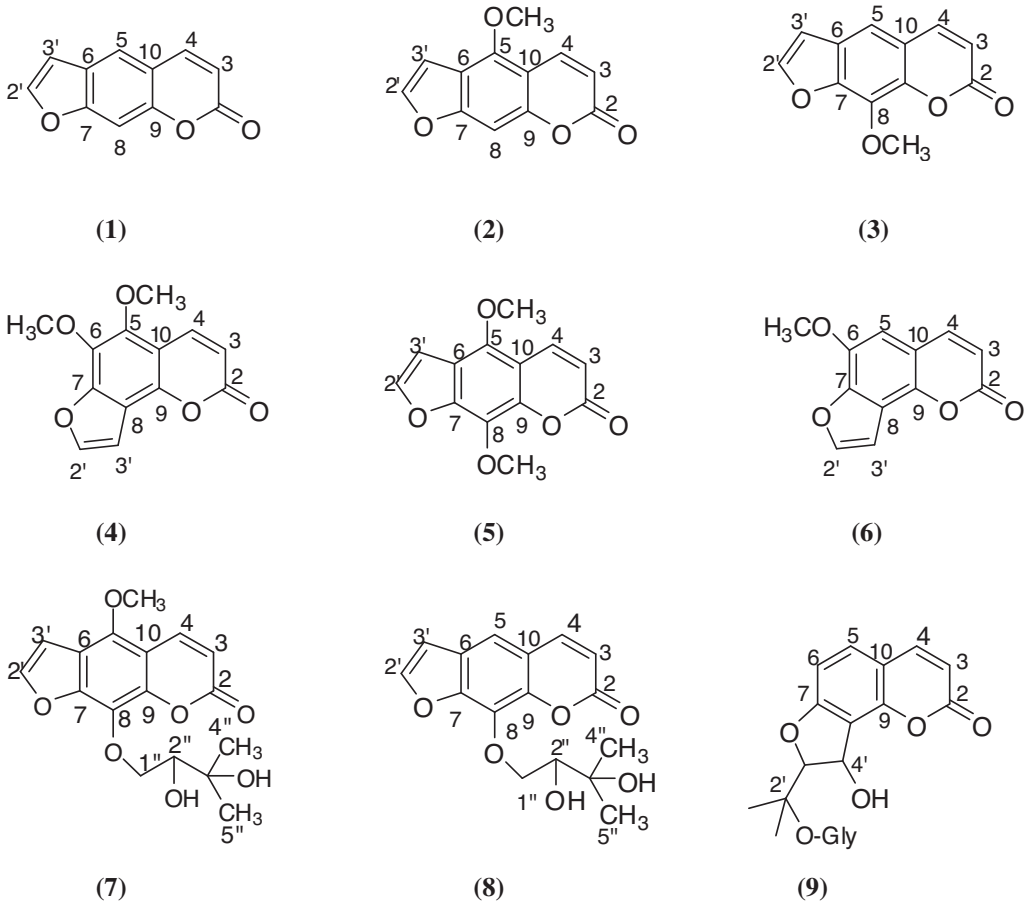


**Figure.** Structures of the compounds.

mass spectroscopic methods as furocoumarins psoralen (**1**),[20] bergapten (**2**),[21] xanthotoxin (**3**),[21] pimpinellin (**4**),[22] isopimpinellin (**5**),[23] sphondin (**6**),[22] byakangelicin (**7**),[24] and heraclenol (**8**),[25] and apterin (**9**)[26] (Figure). Apterin was first isolated from *Zizia aptera*[26] and then from an Apiaceae plant, *Peucedanum japonicum.*[27] From the petroleum ether extract, steroid compound stigmasterol was also obtained.

All coumarins isolated showed purple fluorescence color under UV light (366 nm) and characteristic spectral NMR signals for linear or angular types of furocoumarins. Although these furocoumarins are fairly common in the family Apiaceae plants, including *Heracleum* species, they were isolated here from *H. platytaenium* for the

**Table 1.** $^1$H NMR data of compounds **1**–**9** in $CDCl_3$*, with $J$ in parentheses (Hz).

| Position | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|---|
| 3 | 6.30 d (9.30) | 6.26 d (9.76) | 6.36 d (9.37) | 6.37 d (10.0) | 6.22 d (9.80) | 6.33 d (9.53) | 6.29 d (9.76) | 6.38 d (9.76) | 6.20 d (9.50) |
| 4 | 7.73 d (9.30) | 8.14 d (9.76) | 7.76 d (9.37) | 8.08 d (9.62) | 8.06 d (9.80) | 7.69 d (9.51) | 8.13 d (9.76) | 7.78 d (9.76) | 7.80 d (9.50) |
| 5 | 7.62 s | - | 7.34 s | | - | 6.72 s | - | 7.40 s | 7.46 d (8.59) |
| 6 | | | | | | | | | 6.82 d (8.59) |
| 8 | 7.42 s | 7.13 s | - | - | - | - | - | - | - |
| 2′ | 7.63 d (2.34) | 7.59 d (2.31) | 7.69 d (2.34) | 7.66 d (2.40) | 7.56 d (2.31) | 7.64 d (2.12) | 7.63 d (2.34) | 7.71 d (2.30) | - |
| 3′ | 6.77 d (2.34) | 7.01 d (2.30) | 6.82 d (2.34) | 7.08 d (2.45) | 6.93 d (2.31) | 7.06 d (2.12) | 7.02 d (2.30) | 6.84 d (2.34) | 4.4 d (6.25) |
| $OCH_3$ (C-5) | - | 4.26 s | - | 4.03 s | 4.10 s | - | 4.19 s | - | 5.5 d (6.25) |
| $OCH_3$ (C-6) | - | - | - | 4.14 s | - | 3.98 s | - | - | - |
| $OCH_3$ (C-8) | - | - | 4.27 s | - | 4.11 s | - | - | - | - |
| 1″-$CH_2$ | - | - | - | - | - | - | 4.58 dd (2.73; 0.15) 4.27 dd (8.20; 10.25) | 4.61 dd (2.34; 10.15) 4.41 dd (7.81; 10.15) | - |
| 2″- CH | - | - | - | - | - | - | 3.83 dd (8.21; 2.73) | 3.86 dd (7.81; 2.34) | - |
| 4″-$CH_3$ | - | - | - | - | - | - | 1.32 s | 1.34 s | - |
| 5″-$CH_3$ | - | - | - | - | - | - | 1.28 s | 1.30 s | - |
| Gly-1 | - | - | - | - | - | - | - | - | 4.68 d (7.81) |
| Gly-2 | - | - | - | - | - | - | - | - | 3.03 dd (7.81; 9.6) |
| Gly-3 | - | - | - | - | - | - | - | - | 3.06 dd (9.6; 9.8) |
| Gly-4 | - | - | - | - | - | - | - | - | 3.26 dd, (9.6; 9.8) |
| Gly-5 | - | - | - | - | - | - | - | - | 3.24 m |
| Gly-6 | - | - | - | - | - | - | - | - | 3.38 dd (12.0; 4.3) 3.09 dd (12.0; 2.5) |
| $C2_a'$-$CH_3$ | - | - | - | - | - | - | - | - | 1.50 s |
| $C2_b'$-$CH_3$ | - | - | - | - | - | - | - | - | 1.50 s |

*The assignments were based on APT and HSQC tests.

first time. Among 13 previously investigated *Heracleum* species, bergapten has been obtained from 7 *Heracleum* species, while sphondin was isolated from only 2 *Heracleum* species. The 8 furocoumarins and apterin have never been isolated altogether from any *Heracleum* species. However, they were obtained from *H. platytaenium* in this study. From the isolated furocoumarins, psoralen, bergapten, xanthotoxin, and isopimpinellin have a linearly attached furan ring, while pimpinellin and sphondin have an angular furan ring. Although all of the identified furocoumarins are known compounds, among them, the $^{13}$C NMR data of sphondin and pimpinellin were not previously reported (Tables 1 and 2). As expected, significant chemical shift differences were observed for C-2′ and C-3′ signals as well as C-7 and C-8 signals between angular and linear furocoumarins.

**Table 2.** $^{13}$C NMR data of compounds **2** and **4**–**9** in $CDCl_3$.*

| Position | $\delta$ ($^{13}$C, in $CDCl_3$, 100 MHz) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 2 | 4 | 5 | 6 | 7 | 8 | 9 |
| 2 | 161.42 | 160.83 | 160.46 | 161.03 | 160.16 | 160.16 | 162.01 |
| 3 | 112.55 | 113.73 | 112.89 | 114.49 | 112.86 | 113.84 | 112.30 |
| 4 | 139.21 | 139.90 | 139.38 | 144.35 | 139.47 | 145.23 | 145.01 |
| 5 | 149.57 | 144.45 | 144.01 | 104.58 | 143.93 | 114.85 | 131.02 |
| 6 | 112.51 | 135.12 | 114.83 | 143.02 | 114.46 | 116.47 | 108.10 |
| 7 | 158.32 | 149.81 | 150.03 | 146.96 | 150.16 | 147.94 | 163.20 |
| 8 | 93.85 | 114.11 | 128.22 | 118.58 | 126.05 | 128.50 | 117.50 |
| 9 | 152.71 | 143.25 | 143.01 | 143.13 | 144.88 | 143.33 | 152.60 |
| 10 | 106.02 | 109.44 | 107.67 | 113.61 | 107.44 | 147.94 | 114.50 |
| 2′ | 144.53 | 145.37 | 145.12 | 145.99 | - | - | 78.30 |
| 3′ | 105.02 | 104.31 | 105.07 | 103.77 | - | - | 92.42 |
| 4′ | - | - | - | - | - | - | 69.63 |
| $C_5$-$OCH_3$ | 60.08 | 62.37 | 61.71 | - | 60.71 | - | - |
| $C_6$-$OCH_3$ | - | 61.22 | 60.83 | 56.50 | - | - | - |
| 1″ | - | - | - | - | 76.10 | 75.76 | - |
| 2″ | - | - | - | - | 75.94 | 75.97 | - |
| 3″ | - | - | - | - | 71.51 | 71.51 | - |
| 4″-$CH_3$ | - | - | - | - | 26.71 | 25.07 | - |
| 5″- $CH_3$ | - | - | - | - | 26.71 | 25.03 | - |
| Gly-1 | - | - | - | - | - | - | 98.21 |
| Gly-2 | - | - | - | - | - | - | 73.35 |
| Gly-3 | - | - | - | - | - | - | 76.50 |
| Gly-4 | - | - | - | - | - | - | 70.12 |
| Gly-5 | - | - | - | - | - | - | 77.63 |
| Gly-6 | - | - | - | - | - | - | 60.34 |
| C2$_{a}$′ -$CH_3$ | - | - | - | - | - | - | 23.22 |
| C2$_{b}$′- $CH_3$ | - | - | - | - | - | - | 23.57 |

*The assignments were based on APT, HSQC, and HMBC tests.

This is the first study that reports isolation of furocoumarins **1**–**9** from *H. platytaenium* extracts. In this study, we have investigated antioxidant and anticholinesterase activity of extracts and the isolated coumarins (Tables 3–6). There are some previous studies that reported several of the antioxidant activity results of some furocoumarins. In one of these previous studies, xanthotoxin was investigated by the FRAP method,[28] while isopimpinellin was investigated by DPPH assay[29] and psoralen by DPPH and ABTS methods.[30] However, our study is the first report on the antioxidant activity of pimpinellin and sphondin (Table 3). As anticholinesterase

activity, psoralen, pimpinellin, and sphondin were never investigated before for acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibitory activities (Tables 5 and 6).

**Table 3.** Lipid peroxidation inhibitory activity of the extracts and compounds **(1**–**7**, **9)** by $\beta$-carotene bleaching method (inhibition %).[a]

| Samples (concentration) | 10 $\mu$g/mL | 25 $\mu$g/mL | 50 $\mu$g/mL | 100 $\mu$g/mL |
|---|---|---|---|---|
| *H. platytaenium* PE extract | 49.68 ± 2.35 | 51.10 ± 2.14 | 52.40 ± 2.64 | 54.41 ± 2.84 |
| *H. platytaenium* MeOH extract | 31.52 ± 3.21 | 41.37 ± 3.53 | 48.19 ± 3.62 | 52.27 ± 3.73 |
| 1 | 47.35 ± 2.60 | 50.18 ± 2.57 | 53.08 ± 2.62 | 54.39 ± 2.87 |
| 2 | 29.52 ± 1.65 | 37.74 ± 1.41 | 46.39 ± 1.84 | 49.36 ± 1.92 |
| 3 | 48.87 ± 2.01 | 49.85 ± 2.31 | 47.35 ± 2.86 | 47.62 ± 2.70 |
| 4 | 25.55 ± 0.90 | 33.9 ± 0.85 | 46.21 ± 0.95 | 49.77 ± 1.01 |
| 5 | 30.82 ± 3.02 | 39.48 ± 3.24 | 47.96 ± 3.15 | 49.19 ± 3.12 |
| 6 | 31.56 ± 1.52 | 35.17 ± 1.32 | 45.61 ± 1.47 | 49.69 ± 1.63 |
| 7 | 42.12 ± 2.56 | 43.11 ± 2.34 | 44.41 ± 2.21 | 44.99 ± 2.22 |
| 9 | 47.05 ± 3.04 | 48.49 ± 3.14 | 49.14 ± 3.27 | 51.17 ± 3.65 |
| *BHA | 75.64 ± 0.36 | 76.86 ± 0.61 | 81.22 ± 0.72 | 83.45 ± 0.83 |
| *BHT | 69.49 ± 1.02 | 80.17 ± 1.13 | 81.29 ± 1.35 | 82.69 ± 1.54 |
| *$\alpha$-Toc | 49.05 ± 0.75 | 65.66 ± 0.84 | 70.37 ± 0.91 | 74.29 ± 1.01 |

[a]Values are expressed as means ± SEM of 3 parallel measurements ($P < 0.05$).
*Standard.

**Table 4.** Anticholinesterase activity of the extracts (inhibition %).[a]

| Concentration: 200 $\mu$g/mL | | |
|---|---|---|
| Extracts | AChE | BChE |
| *H. platytaenium* PE | 49.28 ± 1.28 | 56.59 ± 1.62 |
| *H. platytaenium* MeOH | 49.86 ± 1.56 | 65.51 ± 1.63 |
| *Galantamine | 89.02 ± 1.01 | 89.92 ± 1.55 |

[a]Values are expressed as means ± SEM of 3 parallel measurements ($P < 0.05$).
*Standard.

**Table 5.** AChE inhibition (%) activity of the compounds **(1**–**7**, **9)**.[a]

| Concentration | | | | |
|---|---|---|---|---|
| Samples | 25 $\mu$g/mL | 50 $\mu$g/mL | 100 $\mu$g/mL | 200 $\mu$g/mL |
| 1 | 24.47 ± 2.83 | 38.97 ± 2.07 | 42.99 ± 2.89 | 52.74 ± 2.29 |
| 2 | 48.38 ± 1.58 | 54.18 ± 1.90 | 55.72 ± 1.32 | 59.00 ± 1.15 |
| 3 | 43.23 ± 2.99 | 56.25 ± 2.35 | 68.63 ± 3.25 | 73.50 ± 2.62 |
| 4 | 55.21 ± 1.41 | 68.11 ± 0.59 | 70.75 ± 1.21 | 78.57 ± 2.86 |
| 5 | 27.78 ± 1.87 | 45.16 ± 1.93 | 63.5 ± 0.20 | 75.13 ± 0.50 |
| 6 | 63.69 ± 1.27 | 69.66 ± 1.41 | 71.97 ± 2.19 | 75.38 ± 1.04 |
| 7 | 20.33 ± 2.86 | 24.06 ± 2.70 | 31.68 ± 2.31 | 38.21 ± 2.42 |
| 9 | 28.74 ± 1.12 | 36.35 ± 1.81 | 38.79 ± 1.43 | 40.30 ± 1.67 |
| *Galantamine | 49.23 ± 0.62 | 64.19 ± 0.71 | 75.09 ± 0.36 | 89.02 ± 1.01 |

[a]: Values are expressed as means ± SEM of 3 parallel measurements ($P < 0.05$).
*Standard.

**Table 6.** BChE inhibition (%) activity of the compounds **(1–7, 9)**.[a]

| Concentration | | | | |
|---|---|---|---|---|
| Samples | 25 $\mu$g/mL | 50 $\mu$g/mL | 100 $\mu$g/mL | 200 $\mu$g/mL |
| 1 | 5.01 ± 2.17 | 29.79 ± 2.72 | 30.75 ± 2.69 | 36.75 ± 2.19 |
| 2 | 32.72 ± 2.98 | 50.06 ± 2.50 | 54.70 ± 2.06 | 65.01 ± 2.61 |
| 3 | 38.88 ± 3.65 | 51.03 ± 3.56 | 62.75 ± 3.59 | 76.00 ± 2.06 |
| 4 | 78.02 ± 2.61 | 78.63 ± 2.86 | 81.80 ± 2.02 | 82.17 ± 1.66 |
| 5 | 20.94 ± 2.78 | 33.21 ± 2.27 | 40.48 ± 2.01 | 51.22 ± 1.97 |
| 6 | 54.76 ± 1.96 | 60.56 ± 1.36 | 64.77 ± 1.35 | 68.49 ± 1.43 |
| 7 | 41.33 ± 1.95 | 42.25 ± 1.88 | 44.08 ± 1.18 | 53.66 ± 1.92 |
| 9 | 23.99 ± 1.30 | 37.85 ± 1.47 | 44.99 ± 1.86 | 50.48 ± 1.77 |
| *Galantamine | 78.45 ± 1.70 | 79.55 ± 1.06 | 81.75 ± 1.83 | 89.92 ± 1.55 |

[a]Values are expressed as means ± SEM of 3 parallel measurements ($P < 0.05$).
*Standard.

The petroleum ether and methanol extracts of *H. platytaenium* exhibited a very weak DPPH inhibitory activity. The tested furocoumarins (**1–7**, **9**) exhibited almost no free radical inhibition activity, except for pimpinellin (**4**), which showed a weak DPPH inhibitory activity with a value of 16.78% at a concentration of 100 $\mu$g/mL. BHT was used as the standard, which inhibited at 87.42% at the same concentration. All of the tested furocoumarins exhibited moderate lipid peroxidation inhibitory activity (Table 3). The furocoumarins (**1–7**, **9**) were further investigated for their anticholinesterase potential against both AChE and BChE enzymes at 4 concentrations (25, 50, 100, and 200 $\mu$g/mL). Among them, sphondin (**6**) and pimpinellin (**4**) showed highest activity against AChE. Both of them also showed highest activity against BChE. (Tables 5 and 6). There were some anticholinesterase activity screening studies carried out by other researchers, but most of them were carried out at only one concentration for bergapten, xanthotoxin, and isopimpinellin.[12,23] However, we have investigated all of these furocoumarins for anticholinesterase activity at the 4 concentrations, and all of the tested furocoumarins exhibited some anticholinesterase activity against both enzymes. Among them, psoralen was found to be the least active furocoumarin.

## 3. Experimental

### 3.1. Materials and methods

#### 3.1.1. General experimental procedure

$^{1}$H and $^{13}$C NMR, COSY, APT, HMQC, and HMBC spectra were recorded on a Varian Mercury-Mx at 400 MHz for protons and 100 MHz for carbon, with tetramethylsilane (TMS) as an internal standard. Mass spectra Bruker Daltonics MicroTOF Q LC - MS / MS and APCI - ION TRAP Thermo Deca XP Max were used. $\beta$-Carotene, linoleic acid, polyoxyethylene sorbitan monopalmitate (Tween-40), butylated hydroxytoluene (BHT, W218405), butylated hydroxyanisole (BHA, W218308), $\alpha$-tocopherol (47783 Sigma Supelco), 1,1-diphenyl-2-picrylhydrazyl (DPPH), electric eel AChE (type-VI-S, EC 3.1.1.7, 425.84 U/mg), horse serum BChE (EC 3.1.1.8, 11.4 U/mg), 5,5'-dithiobis(2-nitrobenzoic) acid (DTNB), acetylthiocholine iodide, butyrylthiocholine chloride, and galantamine were obtained from Sigma Chemical Co. (Sigma-Aldrich GmbH, Steinheim, Germany). All other chemicals and solvents were of analytical grade. Column chromatography was conducted with silica gel 60 (0.063–0.200 mm), (Merck No: 7734). TLC was performed on silica gel 60 $F_{254}$ plates (Merck No: 5554), and preparative TLC was carried on Merck silica gel 60 $GF_{254}$ plates (Merck No: 7730). Spots and bands were

detected with a UV CAMAG spectrometer (254 and 366 nm).

### 3.2. Plant material

*H. platytaenium* Boiss. was collected and identified by Tuncay Dirmenci from the Ida Mountains (Edremit, Balıkesir) at an altitude of 1100 m (Kazdağı, between Yayla and Kapıkule) in July 2009. A voucher specimen (T.D. 3686) was deposited in the Special Collection of Tuncay Dirmenci at the Biology Department of Balıkesir University, Turkey.

#### 3.2.1. Extraction, fractionation, and isolation

The air-dried aerial parts of the plant (772.5 g) were macerated with light petroleum ether (for 3 days $\times$ 2; each 3L) and subsequently methanol at room temperature (for 6 days $\times$ 2; each 3L). The petroleum ether extract was concentrated under vacuum and the residue was left in a refrigerator (4 °C) for 48 h. By keeping it in the refrigerator, the fat mixture was precipitated. The crude crystalline material of the fat mixture was removed by filtration. The rest of the extract was fractionated on a silica gel column by using first petroleum ether (PE) and then dichloromethane (DCM) with increasing polarity (99:1 $\rightarrow$ 100), and then acetone was gradually added to $CH_2Cl_2$ until 100% and finally methanol (MeOH) was added in the same way. The fractions were compared by TLC and similar fractions were combined to furnish 14 fractions. The combined fractions were separated by repeated preparative TLC procedures to afford 9 furocoumarins and a stigmasterol. The compounds were obtained in the following order: psoralen (**1**) [($CHCl_3$-PE) (8:2) $\times$ 2] (3.5 mg); bergapten (**2**) [(DCM-PE) (8:2) $\times$ 2] (7.8 mg); xanthotoxin (**3**) [(DCM-PE) (8:2) $\times$ 3] (6.2 mg); pimpinellin (**4**) [(DCM-PE) (8:2) $\times$ 3] (7.2 mg); isopimpinellin (**5**) [(DCM-PE) (6:4) $\times$ 2] (7.5 mg); sphondin (**6**) [(DCM-PE) (6:4) $\times$ 4] (6.8 mg); stigmasterol [(DCM-PE) (8:2) $\times$ 3] (4.3 mg); byakangelicin (**7**) [(DCM-acetone) (8:2)] (8.0 mg); and heraclenol (**8**) [(DCM-acetone) (7:3)] (6.2 mg).

The methanol extract (5.0679 g) was concentrated under vacuum and was subjected to column chromatography on silica gel and eluted with dichloromethane, acetone, and methanol with increasing polarity. The collected fractions were applied to preparative TLC and a compound was obtained. Its purification on a silica gel plate [(chloroform-MeOH) (8:2) $\times$ 2] yielded a pure dihydrofurocoumarin glycoside, apterin (**9**) (5.1 mg).

### 3.3. Antioxidant activity

#### 3.3.1. Free radical scavenging activity

The free radical scavenging activity of the extracts and the isolates from *H. platytaenium* was determined by the DPPH assay as described by Blois.[31]

#### 3.3.2. Determination of the antioxidant activity with the $\beta$-carotene bleaching method

The antioxidant activity of the extracts and isolates from *H. platytaenium* was determined using the $\beta$-carotene-linoleic acid model system.[32]

#### 3.3.3. Anticholinesterase activity

AChE and BChE inhibitory activities were measured by modifying the spectrophotometric method developed by Ellman.[33]

### 3.4. Isolated compounds

**Psoralen (1):** White solid powder [$C_{11}H_6O_3$, 186.03], mp 161–162 °C. (+) EI-MS $m/z$ (rel. int.) –25 eV: 187 $[M + H]^+$ (11), 159 (3), 143 (9), 131 (100), 115 (61), 103 (61), 89 (3), 77 (6). $^1$H NMR data in Table 1.

**Bergapten (2):** Colorless pinned crystals [$C_{12}H_8O_4$, 216.04], mp 193–194 °C. El-MS (rel. int., %) $m/z$216 $[M]^+$ (100), 201 $[M - CH_3]^+$ (31.3), 188 $[M - CO]^+$ (11.8), 173 $[MCH_3 - CO]^+$ (58.8), 145 $[M - CH_3 - 2CO]^+$ (26.5), 89 (10.8). $^1$H and $^{13}$C NMR data in Tables 1 and 2.

**Xanthotoxin (3):** Colorless pinned crystals [$C_{12}H_8O_4$, 216.04], mp 146–147 °C. El-MS (rel. int., %) $m/z$216 $[M]^+$ (100), 201 $[MCH_3]^+$ (27.4), 188 $[M - CO]^+$ (9.8), 173 $[M - CH_3 - CO]^+$ (48), 145 $[M - CH_3 - 2CO]^+$ (17.6), 89 (17.8). $^1$H NMR data in Table 1.

**Pimpinellin (4):** Colorless pinned crystals [$C_{13}H_{10}O_5$, 246.21]. EI-MS $m/z$ 246 $[M]^+$ (100), 231 (92), 217 (7), 203 (45), 188 (45), 175 (54), 160 (60), 147 (67), 132 (28), 119 (28), 104 (42), 91 (32), 76 (41), 66 (47). $^1$H and $^{13}$C NMR data in Tables 1 and 2.

**Isopimpinellin (5):** Yellow solid powder [$C_{13}H_{10}O_5$, 246.22], mp 129–130 °C. El-MS (rel. int. %) $m/z$246 $[M]^+$ (96), 231 $[M - CH_3]^+$ (100), 203 $[M - CH_3 - CO]^+$ (15.8), 188 $[M - 2CH_3 - CO]^+$ (4.26), 175 $[MCH_3 - 2CO]^+$ (68.8), 160 $[M - 2CH_3 - 2CO]^+$ (47.5), 147 $[M - CH_3 - 3CO]^+$ (54.9), 132 $[M - 2CH_3 - 3CO]^+$ (20.5), 119 $[M - CH_3 - 4CO]^+$ (19.7), 104 $[M - 2CH_3 - 4CO]^+$ (24.6), 76 $[M - 2CH_3 - 5CO]^+$ (49.2). $^1$H and $^{13}$C NMR data in Tables 1 and 2.

**Sphondin (6):** White solid powder [$C_{12}H_8O_4$, 216.20]. EI-MS $m/z$ 216 $[M]^+$ (100), 201 (50), 188 (30), 173 (52), 159 (2), 157 (2), 145 (38), 129 (5), 116 (5), 108 (9), 101 (7), 95 (15), 89 (24), 79 (16), 74 (15), 63(19). $^1$H and $^{13}$C NMR data in Tables 1 and 2.

**Byakangelicin (7):** Colorless pinned crystals [$C_{17}H_{18}O_7$, 334.32], mp 125–126 °C. $^1$H and $^{13}$C NMR data in Tables 1 and 2.

**Heraclenol (8):** Colorless pinned crystals [$C_{16}H_{16}O_6$, 304.09], mp 117–118 °C. EI-MS $m/z$ 304 $[M]^+$ (54), 289 (21), 245 (15), 229 (4), 215 (29), 202 (100), 186 (11), 174 (69), 157 (11), 145 (32), 129 (19), 118 (15), 102 (9), 89 (56),75 (15), 63 (48), 59 (73). $^1$H and $^{13}$C NMR data in Tables 1 and 2.

**Apterin (9):** White solid powder [$C_{20}H_{24}O_{10}$, 424.30], mp 235–237 °C. APCI-MS (positive): $m/z$ 425 $[M + H]^+$. $^1$H and $^{13}$C NMR data in Tables 1 and 2.

### 3.5. Statistical analysis

The results are the means ± standard deviations of 3 parallel measurements. Analysis of variance was performed using ANOVA procedures. Significant differences between means were determined by Student's t-test, and $P < 0.05$ was regarded as significant.

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