# CHEMICAL CONSTITUENTS FROM THE AQUATIC WEED *Pistia stratiotes*

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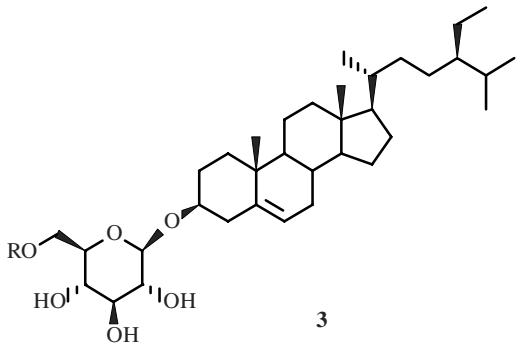
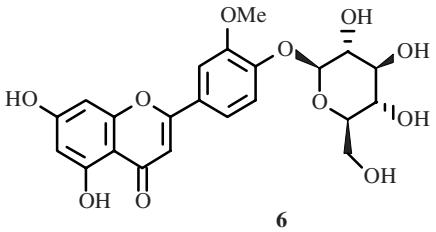
The genus *Pistia* comprises only a perennial species within the family Araceae, namely *Pistia stratiotes* L., commonly known as water lettuce, which is an important pantropical aquatic weed. Moreover, this weed as an exotic invasive plant was distributed mainly in South and East China. Interestingly, this species has the ability to dispel wind-fever, pass through anthema, and is a diuretic [1]. Previous phytochemical studies only lead to the isolation of some stigmastanes from *Pistia stratiotes* [1, 2]. By using column chromatography over silica gel, $Al_2O_3$ gel, and Sephadex LH-20 and reversed-phase RP-18, further fractionation of petroleum ether- and EtOAc-soluble parts of the ethanol extract of *P. stratiotes* afforded eight compounds **1**-**8**. Their structures were determined by spectroscopic analysis (MS, $^1$H NMR, $^{13}$C NMR) as (24*R*)-ergosta-7,22-diene-3$\beta$,5$\alpha$,6$\beta$-triol (**1**), 7$\beta$-hydroxysitosterol (**2**), sitoindoside I (**3**), soya-cerebroside I (**4**), luteolin (**5**), chrysoeriol 4′-*O*-$\beta$-D-glucopyranoside (**6**), $\beta$-sitosterol (**7**), and daucoterol (**8**).




**3**

R = $CH_3(CH_2)_{14}CO$




**6**

**Compound 1**, yield 0.00016 %, colorless needles ($CHCl_3$), $C_{28}H_{46}O_3$, mp 242-244°C, identified as (24*R*)-ergosta-7,22-diene-3$\beta$,5$\alpha$,6$\beta$-triol by comparison of physicochemical data and spectral data (EI-MS, $^1$H NMR and $^{13}$C NMR) with those reported in the literature [3].

**Compound 2,** yield 0.00014 %, colorless needles ($CHCl_3$), $C_{29}H_{48}O_2$, mp 144-145°C, elucidated by comparing physicochemical data and spectral data (EI-MS, $^1$H NMR and $^{13}$C NMR) with those recorded for 7$\beta$-hydroxysitosterol [2, 4, 5].

**Compound 3**, yield 0.0011 %, white powder, $C_{51}H_{90}O_7$, mp 161-163°C. Its spectral data (EI-MS, $^1$H NMR, $^{13}$C NMR) and physicochemical data were identical to those recorded for sitoindoside I in the literature [6].

**Compound 4,** yield 0.0028 %, white amorphous powder, $C_{40}H_{75}NO_9$, mp 180-182°C. FAB-MS, $^1$H NMR, and $^{13}$C NMR data were in good agreement with those reported for soya-cerebroside I [7].

**Compound 5,** yield 0.00027 %, yellow powder, $C_{15}H_{10}O_6$, mp 328~330°C. These data and spectral data (EI-MS, $^1$H NMR, $^{13}$C NMR) were identical with those recorded for an authentic specimen of luteolin [8].

**Compound 6**, yield 0.00013 %, yellow powder, $C_{22}H_{22}O_{11}$, mp 261~263°C. (–)FAB-MS *m/z* 461 $[M-1]^-$, 299 [M-Glc], $^1$H NMR, and $^{13}$C NMR, being in accordance with those of chrysoeriol 4′-*O*-$\beta$-D-glucopyranoside [9].

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**Compound 7,** yield 0.007 %, colorless needles (acetone), mp 138-140°C, identical to those recorded for an authentic specimen of $\beta$-sitosterol, which was characterized.

**Compound 8,** yield 0.00057 %, white amorphous powder (MeOH), mp 285-287°C, identical to those reported for an authentic specimen for daucoterol that was identified.

**General Experimental Procedures**. Melting points were obtained on an XRC-1 apparatus and uncorrected. Optical rotations were measured on a Horiba SEPA-300 polarimeter. NMR spectra were recorded on Bruker AV-400 and DRX-500 spectrometers with TMS as an internal standard, δ in ppm, J in Hz. IR spectra were obtained with a Bruker Tensor 27 FT-IR with KBr pellets. UV spectrum was measured on a Shimadzu double-beam 210A spectrometer. MS (EI, FAB) were recorded with a VG Autospec-3000 spectrometer, *m/z* (rel. int.). ESI and HR-ESI-MS was recorded with an API QSTAR Pulsar 1 spectrometer. Column chromatography (CC) was carried out on silica gel (Qingdao Marine Chemical Ltd., Qingdao, China) and on $Al_2O_3$ (Shanghai Wusi Chemical Reagents Ltd., Shanghai, China) and on Sephadex LH-20 (Amersham Biosciences, Uppsala, Sweden), reverse-phase (RP)-18 (Merck). Toyopearl HW-40F was purchased from Tosoh company.

**Extraction and Isolation.** The dried whole plant of *Pistia stratiotes* L. (7 kg), which was collected near Dianchi, Kunming, Yunnan, China and identified by Prof. Peng H, was extracted with 90% aqueous alcohol under reflux. After filtration and concentration *in vacuo*, the residue was suspended in water and partitioned with petroleum ether and EtOAc. The organic extracts were evaporated to give three parts, namely petroleum ether (195 g) and EtOAc (36 g).

Petroleum ether (195 g) was subjected to $SiO_2$ (200-300 mesh) CC with a gradient elution of $CHCl_3$-MeOH (100 : 0, 95 : 5, 90 : 10, 80 : 20, 70 : 30, 0 : 100), providing (Fr. A-K). Fraction D (26 g) was first subjected to $SiO_2$ CC eluting with petroleum ether–EtOAc (95:5-80:20), then petroleum ether-acetone (95:5), followed by CC on neutral $Al_2O_3$ using petroleum ether-EtOAc (95 : 5→90 : 10) to afford compound **7** (500 mg). Fraction F (9 g) was chromatographed on neutral $Al_2O_3$ column eluted with petroleum ether-EtOAc (90:10→70:30), then $CHCl_3$–MeOH (95:5), and further separated by $SiO_2$ column using petroleum ether–acetone (85:15→70:30), $CHCl_3$–acetone (90:10→70:30), then $CHCl_3$-MeOH (100:0→98:2), then petroleum ether-EtOAc (80 : 20) to produce compound **2** (10 mg). Fraction G (9.5 g) was fractionated by $SiO_2$ column eluting with $CHCl_3$–acetone (90 : 10→70 : 30), $CHCl_3$–EtOAc (70:30), then $CHCl_3$–MeOH (90 : 10), followed by neutral $Al_2O_3$ column with $CHCl_3$–EtOAc (70 : 30) to give compound **3** (80 mg). Fraction H (32 g) was subjected to $SiO_2$ column eluted with $CHCl_3$–acetone (90 : 10→70 : 30), $CHCl_3$–MeOH (95:5), then $CHCl_3$–EtOAc (70:30), then petroleum ether-acetone-$CHCl_3$ (6:3:1), and further separated by neutral $Al_2O_3$ column using $CHCl_3$–MeOH (80:20), providing compounds **1** (11 mg) and **8** (40 mg). Fraction I (15 g) was subjected to $SiO_2$ column with $CHCl_3$-MeOH (95:5→70:30), followed by neutral $Al_2O_3$ column using $CHCl_3$–MeOH (95 : 5→0 : 100), and RP-18 column eluted with MeOH–$H_2O$ ( 90:10) to yield compound **4** (200 mg).

EtOAc extract (36 g) was subjected to CC on $SiO_2$, eluted with step elution of $CHCl_3$–MeOH (100:0, 95:5, 90:10, 80:20, 70 : 30, 60 : 40, 0 : 100), providing 11 fractions (Fr. $A_1$–$A_{11}$). Fraction $A_9$ (2.3 g) was separated by $SiO_2$ column using $CHCl_3$–MeOH–$H_2O$ (90 : 10 : 1), followed by repeated Sephadex LH-20 eluted with MeOH to afford compound **5** (19 mg). Fraction $A_{10}$ (3.1 g) was chromatographed on $SiO_2$ column with $CHCl_3$–MeOH–$H_2O$ (90 : 10 : 1) as elutent, followed by repeated Sephadex LH-20 column (MeOH), and Toyopearl HW-40F column (MeOH), affording compound **6** (9 mg).

## ACKNOWLEDGMENT

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