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CHEMICAL CONSTITUENTS FROM *Tagetes erecta* FLOWERS

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Tagetes erecta belongs to the Compositae family and is grown as an ornamental plant in China. *T. erecta* exhibits nematocidal, fungicidal, and insecticidal activity, and its roots have been used in agriculture for nematode control for a long time [1]. Twenty-two naturally occurring compounds with various carbon skeletons were isolated from the flowers of *T. erecta* by systematic phytochemical investigation, and the structures of the compound were identified by the means of spectroscopic data, including ^{1}H NMR and ^{13}C NMR, and compared with the literature data.

Herein we report the results of a thorough phytochemical study on 22 compounds from the flowers of *T. erecta* by isolation of various fractions of the ethanol extract by silica gel column chromatography. They were *β*-sitosterol (**1**) [2], *β*-daucosterol (**2**) [3], 7*β*-hydroxysitosterol (**3**) [4], lupeol (**4**) [5, 6], erythrodiol (**5**) [7], erythrodiol-3-palmitate (**6**) [8], 1-[5′-(1-propyn-1-yl)-[2,2′-bithiophen]-5-yl]-ethanone (**7**) [9–11], *α*-terthienyl (**8**) [12], quercetagetin (**9**) [13], quercetagetin-7-methyl ether (**10**) [14], quercetagetin-7-*O*-glucoside (**11**) [15], kaempferol (**12**) [16], syringic acid (**13**) [17], gallic acid (**14**) [17], 3-*α*-galactosyl disyringic acid (**15**) [18], 3-*β*-galactosyl disyringic acid (**16**) [18], 6-ethoxy-2,4-dimethylquinoline (**17**) [19], oplodiol (**18**) [20, 21], (3*S*,6*R*,7*E*)-hydroxy-4,7-megastigmadien-9-one (**19**) [22], palmitin (**20**) [23], ethylene glycol linoleate (**21**) [24], and *n*-hexadecane (**22**) [18].

***β*-Sitosterol (1)**. White needle crystals, TLC was identical with that of an authentic sample.

***β*-Daucosterol (2)**. White powder, TLC was identical with that of an authentic sample.

Erythrodiol-3-palmitate (6). White powder. ^{1}H NMR (400 MHz, TMS, $CDCl_3$, δ, ppm, J/Hz): 1.02, 1.67 (2H, 2m, H-1), 1.604, 1.701 (each 1H, m, H-2), 4.48 (1H, dd, J = 6.0, 10.0, H-3), 0.810 (1H, m, H-5), 1.52 (2H, m, H-6), 1.19, 1.43 (each 1H, m, H-7), 1.57, 1.859 (2H, 2m, H-11), 5.18 (1H, t, J = 3.6, H-12), 0.91, 1.45 (2H, 2m, H-15), 1.02, 1.81 (each 1H, m, H-16), 1.95 (1H, br, H-18), 1.11, 1.73 (each 1H, m, H-19), 1.15, 1.21 (each 1H, m, H-21), 1.24, 1.36 (each 1H, m, H-22), 0.85 (3H, s, H-23), 0.915 (3H, s, H-24), 0.929 (3H, s, H-25), 0.83 (3H, s, H-26), 1.137 (3H, s, H-27), 3.19, 3.53 (each 1H, d, J = 11.0, H-28), 0.85 (3H, s, H-29), 0.87 (3H, s, H-30), 2.27 (2H, t, J = 7.5, H-2′), 1.55–1.65 (2H, m, H-3′), 1.22–1.29 (m, H-4′–H-15′), 0.86 (3H, H-16′). ^{13}C NMR (100 MHz, TMS, $CDCl_3$, δ, ppm): 38.185 (C-1), 23.481 (C-2), 80.479 (C-3), 37.704 (C-4), 55.170 (C-5), 18.185 (C-6), 32.439 (C-7), 39.726 (C-8), 47.425 (C-9), 36.887 (C-10), 23.542 (C-11), 122.23 (C-12), 144.162 (C-13), 41.649 (C-14), 25.472 (C-15), 22.680 (C-16), 36.758 (C-17), 42.282 (C-18), 46.349 (C-19), 30.997 (C-20), 34.842 (C-21), 31.905 (C-22), 15.538 (C-23), 27.998 (C-24), 15.55 (C-25), 16.736 (C-26), 25.877 (C-27), 69.698 (C-28), 33.164 (C-29), 23.481 (C-30), 173.752 (C-1′), 34.842 (C-2′), 25.152 (C-3′), 29.02–29.67 (C-4′–C-14′), 22.680 (C-15′), 14.12 (C-16′).

1-[5′-(1-Propyn-1-yl)-[2,2′-bithiophen]-5-yl]-ethanone (7). Light green crystals. ^{1}H NMR (400 MHz, TMS, $CDCl_3$, δ, ppm, J/Hz): 7.11 (1H, d, J = 4.0, H-3), 7.54 (1H, d, J = 4.0, H-4), 7.12 (1H, d, H-3″), 7.04 (1H, d, J = 4.0, H-4″), 2.10 (3H, s, CH_3), 2.525 (3H, s, $COCH_3$). ^{13}C NMR (100 MHz, TMS, $CDCl_3$, δ, ppm): 145.085 (C-2), 124.224 (C-3), 133.243 (C-4), 142.583 (C-5), 136.082 (C-2′), 125.216 (C-3′), 131.992 (C-4′), 125.308 (C-5′), 72.635 (C-1″), 92.505 (C-2″), 4.779 (C-3″), 190.287 (CO), 26.525 (CO-CH_3).

***α*-Terthienyl (8)**. Light green crystals. ^{1}H NMR (400 MHz, TMS, $CDCl_3$, δ, ppm, J/Hz): 7.05 (2H, s, H-3), 7.15 (2H, dd, J = 3.6, 1.0, H-3′), 7.0 (2H, dd, J = 5.1, 3.6, H-4′), 7.24 (2H, dd, J = 5.0, 11.0, H-5′). ^{13}C NMR (100 MHz, TMS, $CDCl_3$, δ, ppm): 137.043 (C-2), 123.621 (C-3), 127.833 (C-4), 124.239 (C-5), 136.1 (C-2′, C-5′), 124.422 (C-3′, C-4′).

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Quercetagetin (9). Yellow powder. ^{1}H NMR (400 MHz, TMS, DMSO-d_6, δ, ppm, J/Hz): 7.663 (1H, s, H-2′), 6.87 (1H, d, J = 8.8, H-5′), 7.52 (1H, d, J = 8.4, H-6′), 6.49 (1H, s, H-8). ^{13}C NMR (100 MHz, TMS, DMSO-d_6, δ, ppm): 146.698 (C-2), 135.390 (C-3), 175.899 (C-4), 145.920 (C-5), 128.599 (C-6), 153.649 (C-7), 93.301 (C-8), 148.873 (C-9), 103.373 (C-10), 122.281 (C-1′), 115.101 (C-2′), 145.103 (C-3′), 147.629 (C-4′), 115.666 (C-5′), 119.992 (C-6′).

Quercetagetin-7-methyl Ether (10). Yellow powder. ^{1}H NMR (400 MHz, TMS, acetone-d_6, δ, ppm, J/Hz): 7.823 (1H, s, H-2), 6.59 (1H, s, H-8), 6.99 (d, J = 8.4, H-5′), 7.70 (1H, d, J = 8.4, H-6′), 3.865 (3H, s, OCH_3). ^{13}C NMR (100 MHz, TMS, acetone-d_6, δ, ppm): 146.865 (C-2), 136.168 (C-3), 176.555 (C-4), 153.756 (C-5), 131.391 (C-6), 157.609 (C-7), 94.201 (C-8), 152.756 (C-9), 104.250 (C-10), 123.478 (C-1′), 115.490 (C-2′), 145.545 (C-3′), 148.101 (C-4′), 115.924 (C-5′), 121.182 (C-6′), 60.445 (OCH_3).

Quercetagetin-7-*O*-glucoside (11). Yellow powder. ^{1}H NMR (400 MHz, TMS, acetone-d_6, δ, ppm, J/Hz): 7.698 (1H, d, J = 2.4, H-2′), 6.89 (1H, d, J = 8.4, H-5′), 7.53 (1H, dd, J = 2.4, 8.4, H-6′), 6.946 (1H, s, H-8), 5.0 (1H, d, J = 3.2, H-1″), 3.74–3.2 (unresolved m, other sugar protons). ^{13}C NMR (100 MHz, TMS, acetone-d_6, δ, ppm): 147.522 (C-2), 129.652 (C-3), 176.158 (C-4), 145.370 (C-5), 129.652 (C-6), 151.612 (C-7), 93.576 (C-8), 148.171 (C-9), 105.136 (C-10), 122.060 (C-1′), 115.406 (C-2′), 145.073 (C-3′), 147.827 (C-4′), 115.984 (C-5′), 119.984 (C-6′), 100.9 (C-1″), 73.2 (C-2″), 75.8 (C-3″), 69.6 (C-4″), 77.3 (C-5″), 60.6 (C-6″).

General. The ^{1}H NMR and ^{13}C NMR spectra of the compounds in $CDCl_3$, acetone-d_6, and DMSO-d_6 were measured on a Varian INOVA-400FT-NMR spectrometer with TMS as internal standard. Silica gel (200–300 mesh, Marine Chemical Factory, Qingdao, China) was used for column chromatography. Sephadex LH-20 was purchased from Pharmacia Company.

Plant Material. The flowers of *T. erecta* were collected in Zhangye City of Gansu Province of China in August 2007. It was identified by Dr. Huan-Yang Qi. Voucher specimens were deposited in the Key Laboratory for Natural Medicine of Gansu Province, Lanzhou Institute of Chemical Physics, Chinese Academy of Sciences, P. R. China.

Extraction, Isolation, and Purification of Compounds. The dried flowers of *T. erecta* (6.3 kg) were powdered and extracted with ethanol (95%) three times (each for 2 hours) at 60°C and concentrated under reduce pressure to give a crude extract (600 g). The ethanol extract (600 g) was placed into 2000 mL water and extracted with petroleum ether and EtOAc to yield two fractions.

The petroleum ether extract (190 g) was subjected to silica gel column chromatography (CC) eluting with petroleum ether (60–90°C)–acetone gradient system (v/v 100:1, 50:1, 30:1, 20:1, 15:1, 10:1, 5:1, 3:1, 1:1 and CH_3OH) to give fractions 1–8 according to TLC analysis. Fraction 2 was subjected to silica gel CC eluting with petroleum ether–acetone (v/v 30:1, 20:1, 10:1, 5:1) to give three subfractions. Further purification of each subfraction through repeated silica gel CC with petroleum ether–acetone (v/v 15:1) and petroleum ether–acetone (v/v 10:1) gave compounds **7** (10 mg), **8** (2 mg), **18** (10 mg), **19** (10 mg), **21** (10 mg), and **6** (10 mg). Fraction 4 was applied to silica gel CC eluting with petroleum ether–acetone (v/v 20:1, 10:1, 5:1) to give fractions 4a–4c. Fraction 4a was purified through repeated silica gel CC with petroleum ether–acetone (v/v 5:1) to give compound **3** (8 mg). Fraction 4b was subjected to silica gel CC eluting with petroleum ether–acetone (v/v 10:1, 5:1) to give compounds **1** (2 g) and **5** (30 mg). Fraction 4c was purified through repeated silica gel CC with petroleum ether–acetone (v/v 5:1) to give compound **20** (10 mg). Fraction 5 was applied to silica gel CC eluting with $CHCl_3$–MeOH gradient system and then through repeated silica gel CC with petroleum ether–acetone (v/v 10:1), $CHCl_3$–MeOH (v/v 5:1), and $CHCl_3$–MeOH (v/v 1:1) to give compounds **4** (10 mg), **10** (10 mg), and **9** (1 g), respectively. Fraction 8 was applied to silica gel CC eluting with $CHCl_3$–MeOH (v/v 10:1, 5:1, 1:1) to give fractions 8a–8c. Fraction 8a was purified through repeated silica gel CC with $CHCl_3$–EtOAc (v/v 5:1) to give compound **17** (10 mg). Fraction 8b was purified through repeated silica gel CC with $CHCl_3$–MeOH (v/v 5:1) to give the compound **2** (1 g). Fraction 8c was subjected to Sephadex LH-20 CC eluting with MeOH to give compound **11** (10 mg). The EtOAc extract (185 g) was applied to silica gel CC eluting with $CHCl_3$–MeOH (v/v 50:1, 30:1, 20:1, 15:1, 10:1, 5:1, 3:1, 1:1 and CH_3OH) to give fractions A–F according to TLC analysis. Fraction E was subjected to silica gel CC eluting with $CHCl_3$–MeOH gradient system to give fractions E_1–E_2. Compound **13** (20 mg) was obtained from fraction E_1 through repeated silica gel CC with petroleum ether–acetone–acetic acid (v/v/v 50:45:6). Fraction E_2 was applied to silica gel CC eluting with petroleum ether–acetone (v/v 10:1) and $CHCl_3$–MeOH (v/v 5:1) to yield compounds **22** (10 mg) and **14** (10 mg) respectively. Fraction F was applied to silica gel CC with the $CHCl_3$–MeOH gradient system to give fractions F_1–F_2. Compound **12** (2 mg) was obtained from fraction F_1 through Sephadex LH-20 CC eluting with $CHCl_3$–MeOH (v/v 1:1), and an inseparable mixture of **15** and **16** (10 mg) was obtained from fraction F_2 through repeated silica gel CC eluting with $CHCl_3$–MeOH (v/v 5:1). The purities of these compounds were determined by spectroscopic analysis.

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