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Research Article

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Chemical research for bioactive constituents in *Kummerowia striata* (Thunb) Schindl

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ABSTRACT

In order to explore the composition of bioactive constituents in Kummerowia striata(Thunb) Schindl, its crude extract was isolated and purified by silica column chromatography, sephadex chromatography and recrystaltization. The chemical structures were determined by related physical properties, spectroscopic data or standard compounds. As a result, eight flavonoids were obtained from Kummerowia striata (Thunb) Schindl and identified as apigenin (1), kaempferol (2), quercetin (3), isoquercitrin (4), rutin (5), luteoloside (6), isovitexin (7) and isoorientin (8), together with β-sitosterol (9). Except apigenin and quercetin, the other seven kinds of compounds were isolated from this plant for the first time.

Key words: *Kummerowia striata (Thun) Schindl*, flavonoids, kaempferol, luteoloside, isoorientin

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INTRODUCTION

The traditional Chinese medicine *Kummerowia striata (Thunb) Schindl*, with wide distribution in China, is also known as 'Jiyancao' [1]. Its principal effects are to clear away heat and detoxicate, diuresis and inhibit diarrhea, so it is usually used for the treatment of traumatic injuries with blood stasis, dysentery, icterohepatitis, sores, carbuncles, furuncles and abscess [2-6]. It is an one-year old plant of Kummerowia striata of Leguminosae, which is used as a folk medicine on inflammation-related therapy for a long time. However, the chemical research on anti-inflammatory ingredients of the plant is almost empty.

In preliminary experiments [7-8] and literature [9], four solution fractions, such as cyclohexane fraction, ethyl acetate fraction, *n*-butanol fraction and aqueous fraction, were obtained from ethanol extract of *Kummerowia striata (Thunb) Schindl*. Furthermore, with Aspirin as positive control, the activities of different solution fractions of the plant were investigated by the method of mice acute ear swelling model. The experimental results have showed that only the ethyl acetate fraction has a significant anti-inflammatory effect [10].

In this study, on the basis of above anti-inflammation research, eight compounds were isolated and purified from the ethyl acetate fraction. They were identified to be apigenin, kaempferol, genistein, quercetin, isoquercitrin, rutin, luteoloside, isovitexin and isoorientin. Among these identified compounds, kaempferol, genistein, isoquercitrin, rutin, luteoloside, isovitexin and isoorientin are isolated from this plant for the first time.

EXPERIMENTAL SECTION

Equipments and Reagents: Melting points were measured using XRC-1 micro-melting point apparatus. MS spectra were measured in Finnigan-TRACE MS, ^{1}H-NMR spectra were measured in DMSO-d_6 on VARIAN INOVA 400M. Column chromatography was carried out on silica gel 100~200 mesh (75~150 μm, Qingdao Haiyang Group

company of China), silica gel 200~300 mesh (45-75μm, Qingdao Haiyang Chemical Group company of China) and sephadex LH-20 (Pharmacia). TLC was performed on a manual silica gel GF254 plate (Qingdao Haiyang Chemical Group of China).

Plant material: *Kummerowia striata (Thunb) Schindl* was collected from the Hongshan District, Wuhan City, Hubei Province, China and identified by Prof. Ke-Li Chen (School of Pharmacy, Hubei University of Chinese Medicine) and stored in the Plant Specimen Department, School of Pharmacy, Hubei University of Chinese Medicine.

Extraction and isolation: The dried plant materials were refluxed repeatedly with 95% EtOH to give a crude extract, which was concentrated under reduced pressure into a thick paste. Amount of water was added with stirring to the suspension, followed by the partition with cyclohexane and ethyl acetate. Then the three parts (cyclohexane, ethyl acetate and water) were dried under reduced pressure.

The ethyl acetate extract was chromatographied on silica gel 100~200 column by using gradient elution with cyclohexane-ethyl acetate, chloroform-methanol. The same subfractions were put together according to the results of TLC analysis. Each part was chromatographied again on silica gel or sephadex repeatedly by using gradient elution with cyclohexane-ethyl acetate, chloroform-methanol. Finally, nine compounds were obtained.

RESULTS AND DISCUSSION

Compound 1: yellow amorphous powders, m. P. 338-341 ^{o}C. The ^{1}H-NMR (400MHz, DMSO-d_6) spectrum displayed δ: 7.93 (2H, d, J=8.5 Hz, H-2′, 6′), 6.92 (2H, d, J=8.5Hz, H-3′, 5′), 6.79 (1H, s, H-3), 6.48 (1H, s, H-8), 6.19 (1H, s, H-6). Above spectral data are consistent with the literature [11], therefore the compound 1 is identified as apigenin.

Compound 2: yellow powders, m. P. 271-274^{o}C. HCl-Mg and $FeCl_3$ reactions were positive. The ESI-MS spectrum displayed its molecular ion peak $[M-H]^{-}$ at m/z 285. The ^{1}H-NMR (400MHz, DMSO--d_6) spectrum displayed δ: 12.46 (1H, s, 5-OH), 10.74 (1H, s, 7-OH), 10.10 (1H, s, 4'-OH), 9.39 (1H, s, 3-OH), 8.04 (2H, d, J=8.8Hz, 2', 6'-H), 6.92 (2H, d, J=8.8Hz, 3', 5'-H), 6.43 (1H, d, J=2.0Hz, 8-H), 6.18 (1H, d, J=2.0Hz, 6-H). The above data were similar to the literature [12], and its spot had the same Rf value with kaempferol on TLC. So the compound 2 is identified as kaempferol.

Compound 3: yellow powders, m. P. 308-311 ^{o}C. HCl-Mg and $FeCl_3$ reactions were positive. The ^{1}H-NMR (400MHz, DMSO-d_6) spectrum displayed δ: 12.48 (1H, s, 5-OH), 10.71 (1H, s, 7-OH), 9.56 (1H, s, 3'-OH), 9.36 (1H, s ,3-OH), 9.29 (1H, s, 4'-OH), 7.66 (1H, d, J=2.0Hz, 2'-H), 7.53 (1H, dd, J=8.6,2.0 Hz, 6'-H), 6.88 (1H, d, J=8.5Hz, 5'-H), 6.39 (1H, d, J=2.0Hz ,8-H), 6.17 (1H, d, J=2.0Hz, 6-H). The above data were identical to the literature [13], and its spot had the same Rf value with quercetin on TLC. Therefore the compound 3 is identified as quercetin.

Compound 4: yellow powders, m. P. 207-209 ^{o}C. HCl-Mg and molish reactions were positive. The D-glucose was detected in its product of acid hydrolysis. ESI-MS spectrum showed: molecular ion peak $[M-H]^{-}$ at m/z 463, and the fragment peak $[M-H-162]^{-}$ at m/z 301, which also proved the existence of glucose fragment. ^{1}H-NMR(400MHz, DMSO-d_6) spectrum displayed δ: 12.66 (1H, s, 5-OH), 7.60 (1H, brd, J=9.0Hz, H-6), 6.86 (1H, d, J=9.0Hz, H-5), 6.42 (1H, d, J=1.8Hz, H-8), 6.21 (1H, d, J=1.8Hz, H-6), 5.49 (1H, d, J=7.5Hz, H-1). Above spectral data were accordance with the literature [14], therefore the compound 4 is identified as isoquercitrin.

Compound 5: yellow powders, m. P. 174-176 ^{o}C. HCl-Mg powder reaction was positive. ^{1}H-NMR (400MHz, DMSO-d_6) spectrum showed δ: 12.59 (C5-OH), 10.89 (C7-OH), 9.66 (C3'-OH), 9.18 (C4'-OH), 7.54 (d, 1H, J=1.8, C2'-H), 7.53 (dd, 1H, J=1.8, 8.5, C6'-H), 6.83 (d, 1H, J=8.5, C5'-H), 6.39 (d, 1H, J=1.4, C8-H), 6.18 (d, 1H, J=1.4, C6-H), 5.34 (d, 1H, J=7.0, C1''-H), 4.51 (brs, 1H, C1'''-H), 3.03~3.70 (m, sugar protons), 0.98 (d, 3H, J = 6.0, C6'''-H). Above spectral data were similar to the literature [15], and its spot had the same Rf value with rutin on TLC. So the compound 4 is identified as rutin.

Compound 6: pale yellow powders, m. P. 254-256 ^{o}C. HCl-Mg and molish reactions were positive. ESI-MS spectrum displayed molecular ion peak $[M-H]^{-}$ at m/z 447, and the fragment peak $[M-H-162]^{-}$ at m/z 285, which also proved the existence of glucose fragment. ^{1}H-NMR (400MHz, DMSO-d_6) spectrum displayed δ: 13.01 (1H,

s ,5-OH), 10.04 (1H, s, 3'-OH), 9.43 (1H, s, 4'-OH), 7.46 (1H, dd, J=8.5, 2.5Hz, H-6'), 7.43 (1H, dd, J=2.5Hz, H-2'), 6.93 (1H, d, J=8.5Hz, H-5'), 6.91 (1H, d, J=2.0Hz, H-8), 6.80 (1H, s, 3-H), 6.46 (1H, d, J=2.0Hz, H-6), 5.46 (1H, d, J=8.0Hz, H-1''), 3.17~3.74 (5H, m, sugar protons). The spot of its acid hydrolysis product had the same Rf value with luteolin on TLC, and ^{1}H-NMR spectral data were consistent with the literature [16] for luteolin-7-O-β-D-glucoside. Therefore, the compound 5 is identified as luteoloside.

Compound 7: pale yellow powders, m. P. 205-207 ^{o}C. HCl-Mg and molish reactions were positive. ESI-MS spectrum showed molecular ion peak $[M-H]^-$ at m/z 431. ^{1}H-NMR (400MHz, DMSO-d_6) spectrum displayed δ: 13.58 (1H, s, 5-OH), 10.81 (1H, s, 7-OH), 10.32 (1H, s, 4'-OH), 7.96 (2H, d, J=8.4Hz, H-2', 6'), 6.95 (2H, d, J=8.4Hz, H-3', 5'), 6.81 (1H, s, H-3), 6.53 (1H, s, H-6), 4.61 (1H, d, J=9.8Hz, H-1''). Above spectral data were accordance with the literature [17], therefore the compound 7 is identified as isovitexin.

Compound 8: Yellow powder, m. P. 241-243 ^{o}C. HCl-Mg powder and molish reactions were positive. ESI-MS spectrum displayed molecular ion peak $[M-H]^-$ at m/z 447 (as same as compound 6), and the fragment peak $[M-H-162]^-$ at m/z 285, which also proved the existence of glucose fragment. ^{1}H-NMR (400MHz, DMSO-d_6) spectrum showed δ: 13.57 (1H, s, 5-OH), 7.43 (1H, d, J=8.0Hz, H-6'), 7.40 (1H, s, H-2'), 6.88 (1H, d, J=8.0Hz, H-5'), 6.68 (1H, s, H-3), 6.48 (1H, s, H-8), 4.59 (1H, d, J=9.7 Hz, H-1'').Above spectral data were similar to the literature [18], therefore the compound 8 is identified as isoorientin.

Compound 9: white needles (water), m. P. 139-141^{o}C. Its colorless spot will become red-violet under heating after sprayed using 5% H_2SO_4 in C_2H_5OH on TLC. ^{1}H-NMR(400MHz, $CDCl_3$) spectrum displayed: δ: 5.29 (1H, d, H-6), 5.06 (1H, -OH), 3.48 (1H, m, H-3). Its melting point did not drop when mixed with β-sitosterol, therefore the compound is determined as β-sitosterol.

As a result, the results of this study discovered the major bioactive constituents in the anti-inflammation fraction in the ethanol extract of *Kummerowia striata (Thunb) Schindl*, which was supposed to be further utilized and developed for various pharmaceutical preparations as a meaningful natural resource in the genus.

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