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Anti-inflammatory Effects of Caper (*Capparis spinosa* L.) Fruit Aqueous Extract and the Isolation of Main Phytochemicals

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Caper (*Capparis spinosa* L.) fruits have been used as food as well as folk medicine in the treatment of inflammatory disorders, such as rheumatism. The present study was carried out to study the anti-inflammatory activities of *C. spinosa* L. fruit (CSF) aqueous extract and to isolate main phytochemicals from its bioactive fractions. The CSF aqueous extract were separated into three fractions (CSF1−CSF3) by macroporous adsorption resins. The fractions CSF2 and CSF3 effectively inhibited the carrageenan-induced paw edema in mice. Systematic fractionation and isolation from CSF2+3 led to the identification of 13 compounds (**1**−**13**). Their chemical structures were elucidated by spectroscopic analyses including nuclear magnetic resonance (NMR) and mass spectrometry (MS) and literature comparisons. Major compounds found in the bioactive fraction CSF2+3 are flavonoids, indoles, and phenolic acids. To our knowledge, 8 of these 13 compounds (**1**−**4**, **6**−**7**, **10**, and **13**) were identified from caper fruits for the first time. The anti-inflammatory effects of these purified compounds are currently under investigation.

KEYWORDS: ***Capparis spinosa*** **L.; anti-inflammatory; flavonoid; indole; phenolic acid**

INTRODUCTION

Caper (*Capparis spinosa* L.) is native to the Mediterranean region and is also widely grown in the dry regions in west and central Asia. Its immature flower buds, unripe fruits, and shoots are consumed as foods or condiments in cooking (*1*). Different parts of this plant, including the flower buds, fruits, seeds, shoots, and bark of roots, were traditionally used as folk medicines in the treatment of disorders, such as rheumatism, stomach problems, headache, and toothache (*2*). Pharmacological studies on this plant revealed that the caper extracts possessed anti-inflammatory effects (*3*−*5*). Although the anti-inflammatory effects of caper have been implicated, the studies are still very limited. Most related papers were published over 15 years ago (*3*−*5*). Several types of compounds, including alkaloids, lipids, flavonoids, indoles, and aliphatic glucosinolates, have been identified from caper (*2*). However, the anti-inflammatory constituents in this plant are largely unknown. Only one study described the isolation of an anti-inflammatory compound, homologous polyprenol cappaprenol-13 (*4*). Identification of natural bioactive compounds from dietary factors or traditional medicines offers great hope in leading compounds for drug development or dietary supplements in treating inflammatory diseases (*6*).

This study was designed to first evaluate anti-inflammatory effects of different fractions of caper fruit extract by carrageenan-induced paw edema in mice. The fractions that showed the inhibitory effects were subjected to further isolation and purification for pure compounds. Our goal was to search for potential bioactive compounds from caper fruit that exhibited anti-inflammatory effects.

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MATERIALS AND METHODS

Chemicals and Reagents. Carrageenan was purchased from Sigma Chemical (St. Louis, MO). Indomethacin was obtained from Shanghai Simpex Pharmaceutical (Shanghai, China). For *in vivo* studies, indomethacin was suspended in 0.5% carboxyl methyl cellulose (CMC) and the prepared solution was stored at 4 °C until use. MeOH, 95% EtOH, petroleum ether, acetonitrile, and $CHCl_3$ were purchased from Shanghai Zhengxing Chemical Plant (Shanghai, China). EtOAc, acetone, and diatomite were obtained from Sinopharm Chemical (Shanghai, China). Silica gel (100−200 mesh) and Sephadex LH-20 were supplied by the Branch of Qingdao Marine Chemical (Qingdao, China) and Shanghai Juyuan Biotechnology (Shanghai, China), respectively.

Plant Material. The fruits of *C. spinosa* were collected in Yili, Xinjiang Uygur Autonomous Region of China, on May 2006. The plant was identified by Dr. Yuan-Jun Xiong (Xinjiang Institute of Chinese Materia Medica and Ethnic Medicines). A voucher specimen (SIPI-060522) was deposited in the Shanghai Institute of Pharmaceutical Industry, Shanghai, China.

General Experimental Procedures. Electrospray ionization−mass spectrometry (ESI−MS) data were measured on a Micromass Q-TOF spectrometer (Milford, MA). Semi-preparative high-performance liquid chromatography (HPLC) was performed with a Waters system consisting of a 510 pump and a 484 UV detector (Milford, MA). ^{1}H and ^{13}C nuclear

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magnetic resonance (NMR) spectra were recorded on a Varian Inova 500 MHz NMR spectrometer (Palo Alto, CA), using tetramethylsilane (TMS) as an internal standard. Thin-layer chromatography (TLC) plates [HSGF-254, thickness of 0.20 mm (10 × 20 cm)] were obtained from Yantai Jangyou Silica Gel Development (Yantai, China). Silica gel (100−200 mesh) was obtained from a branch of the Qingdao Haiyang Chemical Plant (Qingdao, China).

Animals. Male Chinese Kun Ming (KM) mice weighing 18−20 g were purchased from the Shanghai SLAC Laboratory Animal (Shanghai, China). The KM mouse, an outbreed strain of laboratory animal, has been widely used in related pharmaceutical and genetic studies throughout China (*7*). The mice were fed a Purina Lab Diet (autoclave sterilization endurable), purchased from the Naval Medical Research Institute (Shanghai, China). All mice were kept in a specific pathogen-free animal room under the controlled conditions of temperature (23 ± 1 °C) and lighting (12 h light−dark cycle) and provided with a standard laboratory diet and tap water. The animals were allowed to acclimatize to the environment for 1 week before the experiment. All experiments were conducted with the approval of the SIPI Animal Care and Use Committee.

Induction of Acute Inflammation in Mice Hind Paws by Carrageenan. For the inhibitive effect assay, mice were oral administrated with CSF1−CSF3 at 50 and 250 mg/kg that were suspended in 0.5% CMC, 0.5% CMC as a control, or indomethacin at 1 mg/kg as a positive control for 3 days. For CSF1−CSF3, their single maximum tolerated doses (MTDs) were >2 g/kg in mice. A higher dose ($^1/_8$ MTD) and a lower dose ($^1/_{40}$ MTD) were used in the experiment to initially screen the potential anti-inflammatory effects of these fractions. The carrageenan was injected to induce the paw edema 1 h after the last dose on the third day. The right hind paw of each mouse was subcutaneously injected with 10 μL of freshly prepared carrageenan (1.0%, w/v) in physiological saline.

The volumes (mL) of right hind paws of each animal were measured with a YLS-7A plethysmometer from Gene and I Scientific (Beijing, China) 1 h before and 1, 2, 3, 4, 5, and 6 h after carrageenan injection.

The rates of increased paw volume (paw edema) of the right hind paws of mice were calculated by the following equation:

$$\text{paw edema rate (\%)} = (A - B)/B \times 100$$

where A represents the paw volumes at different hours after injection and B represents the paw volume before injection.

The inhibitive rate of *C. spinosa* L. fruit (CSF) on the paw edema was calculated by the following equation:

$$\text{inhibitive rate (\%)} = (\text{Pc} - \text{Pt})/\text{Pc} \times 100$$

where Pc presents the paw edema rate of the negative control group and Pt presents the paw edema rate of CSF extract treatment groups measured at different hours.

Extraction and Isolation. The CSFs (40 kg) were reduced to coarse powder by pulverization, which were then extracted with boiling water (400 L × 2), and each extraction period lasted 2 h. After filtration, the aqueous solution was combined and concentrated under reduced pressure to afford the density of 1.2 g/mL residue. The residue was dissolved in 120 L of 95% ethanol and stored overnight. The ethanol solution was filtered and evaporated under reduced pressure to afford a residue. The residue was subjected to macroporous adsorption resins, washed with water, and eluted by different percentages of ethanol (30, 50, and 70%) for three fractions, successively. The three fractions were concentrated under reduced pressure and then dried in vacuum. CSF1 (30% ethanol elution, 588.0 g), CSF2 (50% ethanol elution, 133.1 g), and CSF3 (70% ethanol elution, 52.2 g) were obtained (**Figure 1**).

For further fractionation and purification, 92.6 g of CSF2+3 (including 66.5 g of CSF2 and 26.1 g of CSF3) was redissolved in 95% EtOH. The ethanol solution was evaporated under reduced pressure to afford a residue, which was subjected to silica gel column chromatography and eluted subsequently with petroleum ether/EtOAc (10:1−1:10), EtOAc, and EtOAc/MeOH (1:1) to afford nine fractions (**Figure 1**).

Fraction 2 was subjected to silica gel column chromatography and eluted with petroleum ether/EtOAc (6:1−1:1), to give nine subfractions. Compounds **10** (R_f = 0.7, 11.2 mg) and **7** (R_f = 0.5, 8.0 mg) were obtained using pre-TLC ($CHCl_3$/MeOH = 10:1) from subfractions 4 and 5. Subfraction 6 was further purified by HPLC. The semi-preparative column

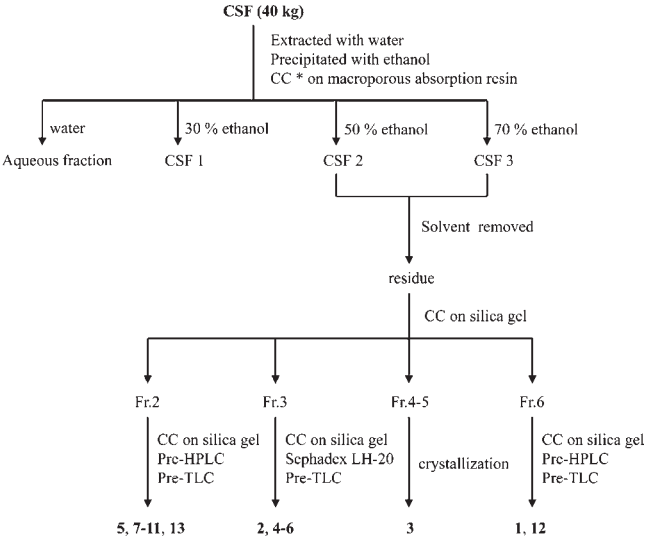

Figure 1. Process of separation of three fractions (CSF1−CSF3) from CSF aqueous extract and further fractionation and isolation of 14 compounds from CSF2+3. CC = column chromatography.

used was a 20 × 300 mm inner diameter, 10 μm, Global C_{18} column (Suzhou, China). Isocratic elution was conducted (MeOH/H_2O = 8:2) for 30 min, detected at 254 nm, to yield compounds **9** (3.0 mg, t_R = 16.6 min) and **13** (3.9 mg, t_R = 20 min). Compound **11** (7.5 mg, t_R = 21.8 min) was obtained from subfraction 7 by HPLC by the same semi-preparative column, with a constant mobile phase (MeOH/H_2O = 1:1) for 30 min and detection at 210 nm. Compounds **5** (R_f = 0.6, 10.8 mg) and **8** (R_f = 0.8, 6.9 mg) were obtained using pre-TLC ($CHCl_3$/MeOH = 15:1) from subfraction 8.

Fraction 3 was subjected to a silica gel column chromatography and eluted with petroleum ether/EtOAc (10:1−1:10), EtOAc, EtOAc/MeOH (1:1), and MeOH to give 25 subfractions. Subfractions 12−14 were purified by repeated Sephadex LH-20 with MeOH and pre-TLC ($CHCl_3$/MeOH = 10:1) to afford compounds **4** (R_f = 0.5, 6.0 mg), **5** (R_f = 0.7, 5.4 mg), and **3** (R_f = 0.6, 8.6 mg). Subfraction 15 was purified by repeated Sephadex LH-20 with MeOH to afford compound **6** (5.5 mg).

Compound **2** (7.0 mg) was obtained by crystallization from fractions 4 and 5.

Fraction 6 was subjected to silica gel column chromatography using petroleum ether/EtOAc (4:1−1:1), EtOAc, and EtOAc/MeOH (1:1) to give 10 subfractions. Subfraction 5 was further separated by HPLC [the same semi-preparative column; constant mobile phase, MeOH/H_2O (containing 0.1% formic acid) (7:3, v/v); detection at 254 nm] to afford compound **1** (9.7 mg, t_R = 22.6 min). Subfraction 3 was purified by pre-TLC ($CHCl_3$/MeOH = 15:1) to compound **12** (R_f = 0.3, 6.9 mg).

Identification of Isolated Compounds. *Flazin* (***1***). Pale yellow powder. ESI−MS *m*/*z*: 309.09 $[M + H]^+$. ^{1}H NMR (500 MHz, DMSO-d_6, δ, ppm; *J*, Hz): 4.68 (2H, s, 2′-CH_2−), 6.62 (1H, s, H-3′), 7.33 (1H, t, *J* = 7.0, H-6), 7.50 (1H, s, H-4′), 7.63 (1H, t, *J* = 7.5, H-7), 7.85 (1H, d, *J* = 7.5, H-8), 8.40 (1H, d, *J* = 7.0, H-5), 8.84 (1H, s, H-4), 11.65 (1H, s, 9-NH). ^{13}C NMR (125 MHz, DMSO-d_6, δ, ppm): 55.9 (2′-CH_2−), 109.1 (C-3′), 110.9 (C-4′), 112.8 (C-8), 115.4 (C-4), 120.4 (C-6), 120.9 (C-4a, C-4b), 121.9 (C-5), 128.7 (C-7), 129.8 (C-3), 131.7 (C-9a), 132.2 (C-1), 141.4 (C-8a), 151.1 (C-5′), 157.2 (C-2′), 166.8 (3-COOH). The ^{1}H and ^{13}C NMR data were consistent with the literature (*8*).

Guanosine (***2***). Pale yellow powder. ESI−MS *m*/*z*: 282.06 $[M - H]^-$. ^{1}H NMR (500 MHz, DMSO-d_6, δ, ppm; *J*, Hz): 3.57 (2H, dd, H-5′), 3.88 (1H, d, *J* = 3, H-4′), 4.10 (1H, d, H-3′), 4.40 (1H, d, *J* = 6, H-2′), 5.70 (1H, d, *J* = 6.5, H-1′), 7.87 (1H, s, H-8). ^{13}C NMR (125 MHz, DMSO-d_6, δ, ppm): 61.5 (C-5′), 70.4 (C-3′), 73.8 (C-2′), 85.3 (C-4′), 86.5 (C-1′), 116.8 (C-5), 135.6 (C-8), 151.3 (C-4), 153.7 (C-2), 156.7 (C-6). The ^{1}H and ^{13}C NMR data were consistent with the literature (*9*).

Capparine A (***3***). Pale yellow powder. ESI−MS *m*/*z*: 279.02 $[M - H]^-$. ^{1}H NMR (500 MHz, DMSO-d_6, δ, ppm; *J*, Hz): 2.50 (3H, s, H-13), 3.74 (3H, s, H-12), 4.36 (1H, d, *J* = 15.0, H-11β), 4.46 (1H, d, *J* = 15.0, H-11α), 6.40 (1H, d, *J* = 1.8, H-7), 6.57 (1H, dd, *J* = 1.8, 8.4, H-5), 7.24 (1H, d,

J = 8.4, H-4), 10.64 (1H, s, NH-1). ^{13}C NMR (125 MHz, DMSO-d_6, δ, ppm): 15.0 (C-13), 55.3 (C-12), 64.2 (C-3), 73.6 (C-11), 96.7 (C-7), 107.6 (C-5), 121.4 (C-9), 125.1 (C-4), 142.4 (C-8), 160.6 (C-6), 161.8 (C-10), 177.5 (C-2). The ^{1}H and ^{13}C NMR data were consistent with the literature (*10*).

Capparine B (***4***). Colorless powder. ESI−MS m/z: 220.07 [M − H]$^-$. ^{1}H NMR (500 MHz, DMSO-d_6, δ, ppm; J, Hz): 2.67 (3H, s, H-12), 3.79 (3H, s, H-11), 6.81 (1H, dd, J = 2.2, 8.8, H-5), 6.92 (1H, d, J = 2.2, H-7), 7.85 (1H, d, J = 8.8, H-4), 12.3 (1H, s, NH-1). ^{13}C NMR (125 MHz, DMSO-d_6, δ, ppm): 16.6 (C-12), 55.3 (C-11), 95.1 (C-7), 111.4 (C-5), 116.0 (C-3), 120.0 (C-9), 120.2 (C-4), 138.2 (C-8), 144.05 (C-2), 156.6 (C-6), 182.9 (C-10). The ^{1}H and ^{13}C NMR data were consistent with the literature (*10*).

1H-Indole-3-carboxaldehyde (***5***). Pale yellow powder. ESI−MS m/z: 144.08 [M − H]$^-$. ^{1}H NMR (500 MHz, DMSO-d_6, δ, ppm; J, Hz): 7.2 (2H, m, H-5, H-6), 7.5 (1H, d, J = 6.4, H-7), 8.0 (1H, d, J = 6.0, H-4), 8.27 (1H, s, H-2), 9.90 (1H, s, H-10), 12.46 (1H, s, H-1). ^{13}C NMR (125 MHz, DMSO-d_6, δ, ppm): 112.6 (C-7), 118.1 (C-3), 120.8 (C-6), 122.1 (C-5), 123.4 (C-4), 124.1 (C-9), 137.1 (C-8), 138.6 (C-2), 185.0 (C-10). The ^{1}H and ^{13}C NMR data were consistent with the literature (*11*).

4-Hydroxy-1H-indole-3-carboxaldehyde (***6***). Pale yellow powder. ESI−MS m/z: 160.12 [M − H]$^-$. ^{1}H NMR (500 MHz, DMSO-d_6, δ, ppm; J, Hz): 6.54 (1H, d, J = 7.6, H-5), 6.92 (1H, d, J = 8, H-7), 7.12 (1H, dd, J = 8, 7.6, H-6), 8.27 (1H, s, H-2), 9.90 (1H, s, CHO-3), 12.20 (1H, s, NH-1). The ^{1}H NMR data were consistent with the literature (*12*).

Chrysoeriol (***7***). Yellow powder. ESI−MS m/z: 299 [M − H]$^-$. ^{1}H NMR (500 MHz, DMSO-d_6, δ, ppm; J, Hz): 3.89 (3H, s, OCH_3-3′), 12.93 (1H, s, OH-5), 6.20 (1H, d, J = 2.0, H-6), 6.50 (1H, d, J = 2.0, H-8), 6.85 (1H, s, H-3), 6.91 (1H, d, J = 8.8, H-5′), 7.52 (1H, d, J = 2.0, H-2′), 7.54 (1H, dd, J = 8.8, 2.0, H-6′). The ^{1}H NMR data were consistent with the literature (*13*).

Apigenin (***8***). Yellow powder. ESI−MS m/z: 269.01 [M − H]$^-$. ^{1}H NMR (500 MHz, DMSO-d_6, δ, ppm; J, Hz): 6.12 (1H, s, H-6), 6.40 (1H, s, H-3), 6.69 (1H, s, H-8), 6.92 (2H, d, J = 8.4, H-3′, H-5′), 7.88 (2H, d, J = 8.4, H-2′, H-6′). The ^{1}H NMR data were consistent with the literature (*14*).

Kaempferol (***9***). Yellow powder. ESI−MS m/z: 285.06 [M − H]$^-$. The compound was compared to the authentic standard and identified as kaempferol.

Thevetiaflavone (***10***). Yellow powder. ESI−MS m/z: 283 [M − H]$^-$. ^{1}H NMR (500 MHz, DMSO-d_6, δ, ppm; J, Hz): 3.85 (3H, s, OCH_3-5), 6.32 (1H, s, H-6), 6.72 (2H, s, H-8, H-3), 6.87 (2H, d, J = 8.4, H-3′, H-5′), 7.87 (2H, d, J = 8.8, H-2′, H-6′). The ^{1}H NMR data were consistent with the literature (*15*).

5-Hydroxymethylfuraldehyde (***11***). Pale yellow oily liquid. ES−MS m/z: 125.06 [M − H]$^-$. ^{1}H NMR (500 MHz, DMSO-d_6, δ, ppm; J, Hz): 4.50 (2H, s, H-6), 5.58 (1H, s, 6-OH), 6.60 (1H, d, J = 4, H-4), 7.49 (1H, d, J = 4, H-3), 9.54 (1H, s, 2-CHO). ^{13}C NMR (125 MHz, DMSO-d_6, δ, ppm): 55.9 (C-6), 109.7 (C-4), 124.2 (C-3), 151.7 (C-5), 162.2 (C-2), 178.0 (2-CHO). The ^{1}H and ^{13}C NMR data were consistent with the literature (*16*).

Vanillic Acid (***12***). White powder. ES−MS m/z: 167.06 [M − H]$^-$. ^{1}H NMR (500 MHz, DMSO-d_6, δ, ppm; J, Hz): 3.79 (3H, s, 3-OCH_3), 6.82 (1H, d, J = 9.0, H-5), 7.41 (1H, s, H-2), 7.42 (2H, d, J = 9.0, H-6). ^{13}C NMR (125 MHz, DMSO-d_6, δ, ppm): 55.5 (3-OCH_3), 112.7 (C-5), 114.9 (C-2), 122.9 (C-1), 123.3 (C-6), 147.1 (C-3), 150.7 (C-4), 167.7 (1-COOH). The ^{1}H and ^{13}C NMR data were consistent with the literature (*17*).

Cinnamic Acid (***13***). Whiter powder. ES−MS m/z: 149.08 [M + H]$^+$. ^{1}H NMR (500 MHz, DMSO-d_6, δ, ppm; J, Hz): 6.55 (1H, d, J = 16, H-2), 7.41 (3H, m, H-5, H-7, H-9), 7.56 (1H, d, J = 16, H-3), 7.67 (2H, m, H-6, H-8). ^{13}C NMR (125 MHz, DMSO-d_6, δ, ppm): 119.4 (C-2), 127.8 (C-5, C-9), 128.6 (C-6, C-8), 129.8 (C-7), 134.0 (C-4), 143.2 (C-3), 167.5 (1-COOH). The ^{1}H and ^{13}C NMR data were consistent with the literature (*18*).

Statistical Analysis. For *in vivo* studies, all values are expressed as mean ± standard error of the mean (SEM). Statistical significance of the differences between groups was assessed by the t test using SigmaStat (version 3.5). $p < 0.05$ was considered significant.

RESULTS AND DISCUSSION

CSF Inhibits the Carrageenan-Induced Paw Edema of Mice by Oral Treatment. In an earlier paper, an aqueous extract of *C. spinosa* was found to possess significant anti-inflammatory activity against carrageenan-induced edema in rats (*3*). Hence, caper fruit aqueous extract was chosen in this study to search for anti-inflammatory compounds. The carrageenan-induced paw edema, which is the most commonly used animal model of inflammation and has been accepted as a useful phlogistic tool for the investigation of new anti-inflammatory agents (*19*), was used to evaluate anti-inflammatory effects of the three fractions of caper fruit aqueous extract. The aqueous extract was separated into three fractions, CSF1, CSF2, and CSF3, by macroporous adsorption resins eluted by different percentages of EtOH.

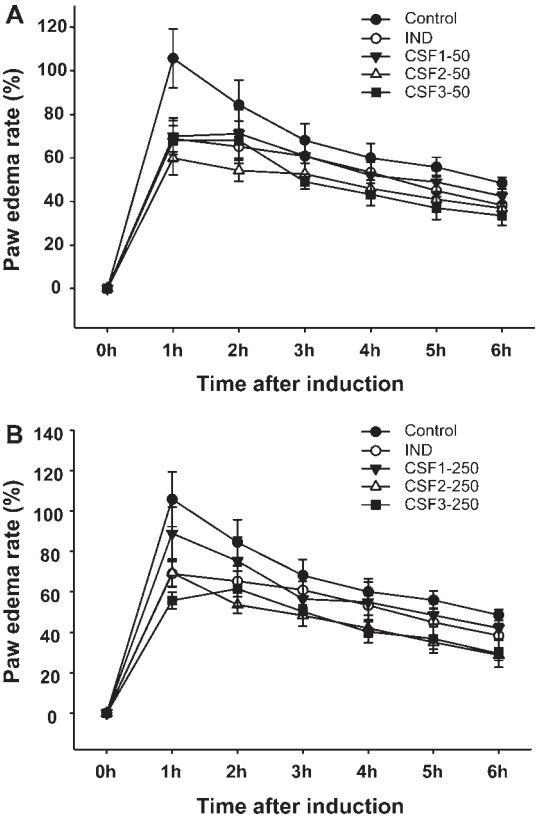

Figure 2. Inhibition of carrageenan-induced paw edema of mice by oral treatment of CSF1, CSF2, and CSF3 for 3 days prior to the induction at doses of 50 mg/kg (A, CSF1-50, CSF2-50, and CSF3-50) and 250 mg/kg (B, CSF1-250, CSF2-250, and CSF3-250). Indomethacin (IND) was also administrated orally at 1 mg/kg for 3 days as a positive control. The carrageenan was injected 1 h after the last dose. Data are expressed as mean ± SEM (n = 6).

The inflammatory patterns of carrageenan-induced paw edema of mice as well as the inhibitory effects of CSF1−CSF3 on this inflammatory model were shown in **Figure 2**. The inflammation induced by carrageenan was observed at 3 h after the injection, and the edema lasted until the end of the experiment (6 h). CSF1 at 50 and 250 mg/kg failed to inhibit the edema in mice with an inhibitive rate of only 12.3 and 13.3% (not significant) at 6 h. CSF2 and CSF3 showed inhibitory effects in this model. The inhibitory rates for CSF2 at 50 and 250 mg/kg at 6 h after induction were 24.0% ($p < 0.05$) and 40.8% ($p < 0.01$), respectively. CSF3 showed similar inhibitory effects as 31.0% ($p < 0.05$) at 50 mg/kg and 39.3% ($p < 0.05$) at 250 mg/kg. The inhibitory effect of positive control indomethacin was 20.9% (not significant).

In the follow-up experiment, CSF2 and CSF3 (CSF2+3) were combined to test its inhibitory effect in the same mice model. The inhibitory rate at 6 h after induction for CSF2+3 at 50 and

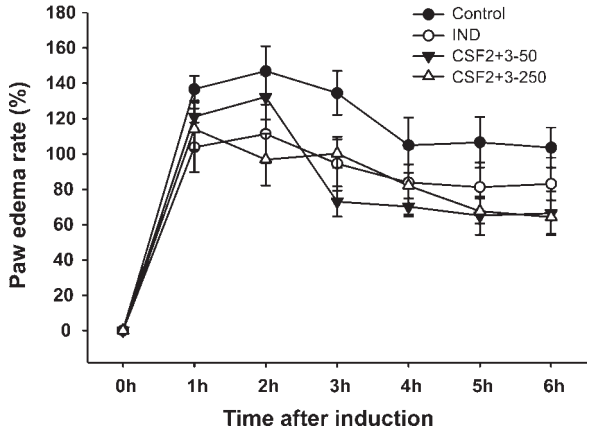

Figure 3. Inhibition of carrageenan-induced paw edema of mice by oral treatment of CSF2+3 for 3 days prior to the induction at doses of 50 mg/kg (CSF2+3-50) and 250 mg/kg (CSF2+3-250). Indomethacin (IND) was also administrated orally at 1 mg/kg for 3 days as a positive control. The carrageenan was injected 1 h after the last dose. Data was expressed as mean ± SEM (n = 10).

250 mg/kg was 38.9% ($p < 0.05$) and 36.6% ($p < 0.05$), whereas the inhibitory effect of indomethacin was 23.8% (not significant) (**Figure 3**).

These results indicated that the anti-acute inflammatory effects of CSF2 and CSF3 in mice were dose-dependent. The maximum phlogistic response of carrageenan was observed at 5−6 h after the injection in the vehicle-treated animals, suggesting that CSF2 and CSF3 may act in later phases of inflammation.

In above-mentioned experiments, CSF2 and CSF3 were found to significantly inhibit acute paw edema evoked by carrageenan injection. Interestingly, CSF2 and CSF3 were eluted from macroporous resin with 50 and 70% ethanol, which indicated that these two fractions contained relatively less polar compounds compared to CSF1. This suggested that, although the aqueous extract of *C. spinosa* was found to possess significant anti-inflammatory activity (*3*), the actual bioactive compounds may actually be less polar compounds. In future studies, it may be more productive to extract with alcohol rather than using an aqueous extract in identifying anti-inflammatory compounds.

Characterization of Compounds. Systematic fractionation and isolation were conducted in the combined fraction of CSF2+3, which was regarded as the main bioactive fraction. A total of 13 compounds were obtained from this combined fraction. Their structures were elucidated by ESI−MS and ^{1}H and ^{13}C NMR spectra and a comparison to literature. They were identified as flazin (**1**), guanosine (**2**), capparine A (**3**), capparine B (**4**), 1*H*-indole-3-carboxaldehyde (**5**), 4-hydroxy-1*H*-indole-3-carboxaldehyde (**6**), chrysoeriol (**7**), apigenin (**8**), kaempferol (**9**), thevetiaflavone (**10**), 5-hydroxymethylfuraldehyde (**11**), vanillic acid (**12**), and cinnamic acid (**13**) (**Figure 4**). To our knowledge, eight compounds (**1**−**4**, **6**−**7**, **10**, and **13**) were identified from CSFs for the first time. Notably, flazin (**1**), a β-carboline compound, represented a compound class that has not been reported from CSFs. Although it is impossible to pinpoint the exact bioactive constituent(s) from the 13 compounds at this stage, these compounds are excellent candidates in future studies in identifying anti-inflammatory compounds. Indeed, the flavonoids, phenolic acids, indoles, and their analogue compounds have all been implicated as anti-inflammatory agents from various plants (*20*−*29*). It is also possible that anti-inflammatory activities of CSF aqueous extract bioactive fractions came from synergistic effects combining flavonoids, indoles, and phenolic acids. The activities of these

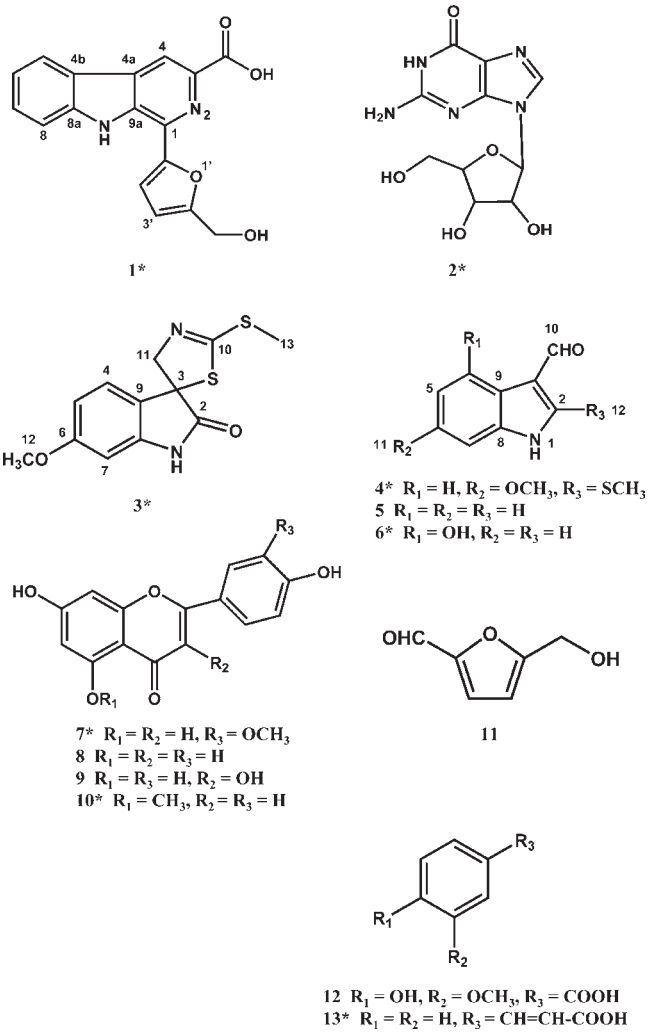

Figure 4. Chemical structures of 14 compounds isolated from caper fruit aqueous extract bioactive fractions of CSF2+3 (∗ indicates compounds reported from caper fruits for the first time).

purified compounds and the underlying mechanism are currently being investigated in our laboratory.

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LITERATURE CITED

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