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# Biflavonoids from Caper (*Capparis spinosa* L.) Fruits and Their Effects in Inhibiting NF-kappa B Activation

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**ABSTRACT:** Caper (*Capparis spinosa* L.) fruits have been widely used as food and folk medicine in the Mediterranean basin and in central and west Asia. In this study, two biflavonoids, isoginkgetin, and ginkgetin, together with three other flavonoids, were isolated from caper fruits. Their chemical structures were elucidated by spectroscopic analyses and comparison with literature. To our knowledge, isoginkgetin, ginkgetin and sakuranetin were identified in caper for the first time. Notably, it is also the first time that biflavonoids have ever been found in the Capparidaceae. Concentrations of the two biflavonoids were measured in caper fruits collected from four major growing areas in northwest China. The anti-inflammatory effects of the flavonoids from caper fruits were evaluated by secreted placental alkaline phosphatase (SEAP) reporter assay, which was designed to measure nuclear factor-kappa B (NF-$\kappa$B) activation. Isoginkgetin and ginkgetin showed inhibitory effects in initial screen at 20 $\mu$M, while the effect of ginkgetin was much greater than that of isoginkgetin. In a dose−response experiment, the $IC_{50}$ value of ginkgetin was estimated at 7.5 $\mu$M, suggesting it could be a strong NF-$\kappa$B inhibitor and worthy of study in vivo.

**KEYWORDS:** caper, *Capparis spinosa*, ginkgetin, NF-$\kappa$B, SEAP reporter assay

## INTRODUCTION

Caper (*Capparis spinosa* L.) is a small shrub belonging to the family Capparidaceae. It is widely grown in the Mediterranean region and the drier climates found regions in west and central Asia. Different parts of this plant, such as the flower buds, fruits, seeds, shoots and bark of roots, have been used as food or folk medicines.[1,2] Caper has been found to exhibit diverse bioactivities including anti-inflammatory effects.[3−5] In our previous study, anti-inflammatory effects of different fractions of caper fruit aqueous extract were evaluated by inhibiting the carrageenan-induced paw edema of mice.[6] Systematic fractionation and isolation from the bioactive fractions led to the identification of 13 compounds, and four of them were flavonoids. Remarkably, three of these four compounds (chrysoeriol, apigenin, and kaempferol) have been reported elsewhere to inhibit nuclear factor-kappa B (NF-$\kappa$B) activation,[7−9] which is considered as an important underlying mechanism of the anti-inflammatory activities of flavonoids.[10] The NF-$\kappa$B transcription factor plays a critical role in various cellular processes associated with innate and adaptive immune responses, as well as cell proliferation, death and development.[11] In relation to diseases common to mammals, NF-$\kappa$B has been shown to play an important role in cardiovascular diseases[12] and cancer.[13]

The objective of this study is to isolate additional flavonoids from bioactive fractions of caper fruits extract according to the previous study[6] and to evaluate the activities of these flavonoids as potential NF-$\kappa$B inhibitors. The inhibitory activities of flavonoids were evaluated by secreted placental alkaline phosphatase (SEAP) reporter assay, conducted in RAW-Blue cells that were derived from RAW 264.7 macrophages. They stably expressed the SEAP gene inducible by NF-$\kappa$B. Our goal is to identify the actual bioactive compounds in caper fruits that produce anti-inflammatory bioactivities.

## MATERIALS AND METHODS

**Chemicals and Reagents.** Silica gel (100−200 mesh) was obtained from Qingdao Haiyang Chemical Plant (Qingdao, China). Sephadex LH-20 was obtained from Shanghai Juyuan Biotechology Corporation (Shanghai, China). Solvents for high-performance liquid chromatography (HPLC) were HPLC grade and obtained from Shanghai Xingke Biochemicals (Shanghai, China). All other solvents used in extraction and isolation were obtained from Sinopharm Chemical Reagent (Shanghai, China)

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

For HPLC comparisons, the fruits of *C. spinosa* were collected again on May 2009 from four major growing areas in the Xinjiang Uygur Autonomous Region of China, including Yili, Hami, and two different locations in Hetian (Hetian 1 and Hetian 2).

After harvest, the caper fruits were allowed to dry in open air in the shaded area. The dried fruits were stored at 4 °C until analysis.

**Instrumentaion.** Electrospray ionization mass spectrometry (ESIMS) data were measured on Micromass Q-TOF spectrometer (Milford, MA). $^{1}H$ and $^{13}C$ NMR spectra were recorded using a Varian Inova 400 MHz NMR spectrometer (Palo Alto, CA) using tetramethylsilane (TMS) as an internal standard. HPLC analyses were conducted in an Agilent HPLC 1100 Series

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system (Agilent, Palo Alto, CA) consisting of a degasser, a quaternary pump, an autosampler, a column oven, and a UV detector.

**Extraction and Isolation Procedure.** The caper fruits (30 kg) were extracted with boiling water (80 L × 3) for 2 h and precipitated with ethanol-$H_2O$ (7:3, v/v) overnight. The solution was separated from precipitates by filtration. The filtrate was evaporated under reduced pressure to afford a residue, which was then suspended in water and partitioned with petroleum ether, chloroform, ethyl acetate, and *n*-butanol successively. After solvent evaporation under reduced pressure, four semidried fractions were obtained: petroleum ether fraction (40 g), chloroform fraction (168 g), ethyl acetate fraction (213 g), and *n*-butanol fraction (300 g).

A portion of chloroform fraction (10 g) was subjected to polyamide column chromatography (column size 2 L) eluted with ethanol-$H_2O$ (30%, 50%, 70%, and 95% ethanol successively, with 3 L each). The 95% ethanol fraction was further purified by Sephadex LH-20 with MeOH to afford 30 fractions. Fr.11−30 was subjected to silica gel (100−200 mesh) column chromatography (column size 500 mL) using petroleum ether/EtOAc (gradient, 6:1−1:1) to give 90 subfractions. Sub-Fr.25−40 was purified by Sephadex LH-20 with MeOH to yield compound **5** (7.6 mg). Sub-Fr.63−80 was purified by preparative HPLC (Global $C_{18}$, 2.5 × 300 mm; mobile phase, MeOH/$H_2O$ (9:1, v/v); detection at 270 nm) to afford **1** (9.1 mg, $t_R$ = 23.9 min) and **2** (17.6 mg, $t_R$ = 26.3 min).

A portion of *n*-butanol fraction (30 g) was subjected to silica gel column chromatography (column size 3 L) and eluted with $CHCl_3$-MeOH (gradient, 100:1−2:1) to give 118 fractions. Fr.39−50 were further separated with Sephadex LH-20 using MeOH to afford compound **3** (8.3 mg) and compound **4** (9.6 mg).

**Identification of Isolated Compounds.** *Isoginkgetin* (**1**), yellow crystal, mp 349−351 °C (reported value 355 °C [14]); ESIMS: $m/z$ = 565.16 $[M-H]^-$; $^1$H NMR (DMSO-$d_6$) ppm: $\delta$ 3.75 (3H, s, 4‴-$OCH_3$), 3.78 (3H, s, 4′-$OCH_3$), 6.20 (1H, s, H-6), 6.42 (1H, s, H-6″), 6.49 (1H, s, H-8), 6.90 (1H, s, H-3″), 6.92 (1H, s, H-3), 6.94 (2H, d, $J$ = 8.5 Hz, H-3‴, 5‴), 7.36 (1H, d, $J$ = 10.0 Hz, H-5′), 7.61 (2H, d, $J$ = 8.5 Hz, H-2‴, 6‴), 8.05 (1H, s, H-2′), 8.18 (1H, d, $J$ = 10.0 Hz, H-6′), 12.92 (1H, s, 5-OH), 13.06 (1H, s, 5′−OH); $^{13}$C NMR (DMSO-$d_6$) ppm: $\delta$ 55.5 (4′-$OCH_3$), 56.0 (4‴-$OCH_3$), 94.2 (C-8), 98.7 (C-6″), 98.9 (C-6), 103.3 (C-3″), 103.7 (C-3), 103.8 (C-10, 8″, 10″), 111.8 (C-5′), 114.6 (C-3‴, 5‴), 121.6 (C-3′), 122.6 (C-1′), 122.9 (C-1‴), 127.8 (C-2‴, C-6‴), 128.3 (C-6′), 130.9 (C-2′), 154.5 (C-9″), 157.5 (C-9), 160.5 (C-5″), 160.7 (C-4′), 161.5 (C-7″), 161.8 (C-5), 162.3 (C-4‴), 163.1 (C-7), 163.4 (C-2), 164.3 (C-2″), 181.8 (C-4), 182.01 (C-4″). The data were consistent with the known compound.[15,16]

*Ginkgetin* (**2**), yellow crystal, mp 347−349 °C (reported value 342−344 °C [14]); ESIMS: $m/z$ = 565.18 $[M-H]^-$; $^1$H NMR (DMSO-$d_6$) ppm: $\delta$ 3.77 (3H, s, 4′-$OCH_3$), 3.81 (3H, s, 7-$OCH_3$), 6.21 (1H, s, H-6), 6.34 (1H, s, H-6″), 6.68 (1H, s, H-3″), 6.70 (2H, d, $J$ = 8.5 Hz, H-3‴, 5‴), 6.77 (1H, s, H-8), 6.96 (1H, s, H-3), 7.32 (1H, d, $J$ = 9.0 Hz, H-5′), 7.46 (2H, d, $J$ = 8.5 Hz, H-2‴, 6‴), 8.04 (1H, d, $J$ = 2.0 Hz, H-2′), 8.15 (1H, dd, $J$ = 9.0, 2.0 Hz, H-6′), 12.92 (1H, s, 5-OH), 13.04 (1H, s, 5″−OH); $^{13}$C NMR (DMSO-$d_6$) ppm: 55.9 (4′-$OCH_3$), 56.1 (7-$OCH_3$), 92.7 (C-8), 98.2 (C-6″), 99.5 (C-6), 102.2 (C-3″), 103.7 (C-3, C-10″), 103.9 (C-8″), 104.8 (C-10), 111.6 (C-5′), 115.9 (C-3‴, C-5‴), 121.3 (C-1‴), 122.2 (C-1′), 123.1 (C-3′), 127.8 (C-6′, C-2‴, C-6‴), 131.2 (C-2′), 154.5 (C-9″), 157.4 (C-9), 160.6 (C-4′, C-5″), 161.0 (C-4‴), 161.2 (C-5), 161.7 (C-7″), 163.9 (C-2, C-2″), 165.2 (C-7), 181.4 (C-4), 182.0 (C-4″). The data were consistent with the known compound.[15,16]

*Kaempferol-3-O-rutinoside* (**3**), yellow crystal, mp 203−204 °C (reported value 204−208 °C [17]); ESIMS: $m/z$ = 593 $[M-H]^-$; $^1$H NMR (DMSO-$d_6$) ppm: $\delta$ 1.00 (3H, d, $J$ = 6.4 Hz, Rha-6-$CH_3$), 4.38(1H, d, $J$ = 1.2 Hz, Rha-H-1), 5.30 (1H, d, $J$ = 7.2 Hz, Glc-H-1), 6.19 (1H, d, $J$ = 2.4 Hz, H-6), 6.40 (1H, d, $J$ = 2.4 Hz, H-8), 6.86 (2H, d, $J$ = 9.2 Hz, H-3′, 5′), 7.98 (2H, d, $J$ = 9.2 Hz, H-2′, 6′), 12.53 (1H, s, 5-OH); $^{13}$C NMR (DMSO-$d_6$) ppm: 17.8 (C-6‴), 66.9 (C-6″), 68.3 (C-5‴), 70.0 (C-4″), 70.4 (C-2‴), 70.6 (C-3‴), 71.8 (C-4‴), 74.2 (C-2″), 75.8 (C-5″), 76.4 (C-3″), 93.8 (C-8), 98.8 (C-6), 100.8 (C-1‴), 101.4 (C-1″), 104.0 (C-10), 115.2 (C-3′, C-5′), 120.9 (C-1′), 130.9 (C-2′, C-6′), 133.2 (C-3), 156.5 (C-2), 156.9 (C-9), 159.9 (C-4′), 161.2 (C-5), 164.2 (C-7), 177.4 (C-4). The data were consistent with the known compound.[18]

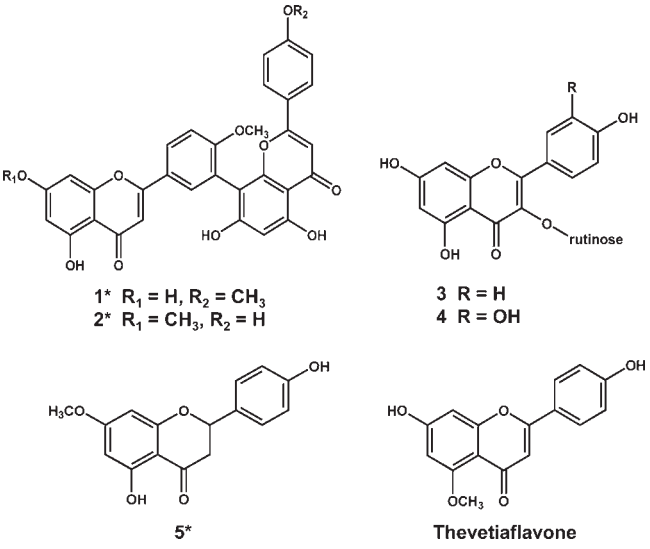


**Figure 1.** The structures of five flavonoids isolated from caper fruits in the present study. The corresponding compound names for **1**−**5** are isoginkgetin, ginkgetin, kaempferol-3-*O*-rutinoside, quercetin-3-*O*-rutinoside and sakuranetin, respectively (* indicates compounds reported from caper fruits for the first time). Thevetiaflavone was also isolated from caper fruits reported in the previous study.[6]

*Quercetin-3-O-rutinoside* (**4**), yellow crystal, mp 180−182 °C (reported value 182−186 °C [17]); ESIMS: $m/z$ = 609 $[M-H]^-$; $^1$H NMR (DMSO-$d_6$) ppm: $\delta$ 1.02 (3H, d, $J$ = 6.0 Hz, Rha-$CH_3$), 4.39 (1H, d, $J$ = 1.2 Hz, Rha-H-1′), 5.34 (1H, d, $J$ = 7.6 Hz, Glc-H-1′), 6.19 (1H, d, $J$ = 2.0 Hz, H-6), 6.38 (1H, d, $J$ = 2.0 Hz, H-8), 6.84 (1H, d, $J$ = 9.2 Hz, H-5′), 7.52 (1H, d, $J$ = 2.4 Hz, H-2′), 7.54 (1H, dd, $J$ = 9.2, 2.4 Hz, H-6′), 12.58 (1H, s, 5-OH); $^{13}$C NMR (DMSO-$d_6$) ppm: 17.8 (C-6‴), 67.0 (C-6″), 76.0 (C-5″), 76.5 (C-3″), 93.6 (C-8), 68.3 (C-5‴), 70.0 (C-4″), 70.4 (C-2‴), 70.6 (C-3‴), 71.9 (C-4‴), 74.1 (C-2″), 98.7 (C-6), 100.8 (C-1‴), 101.2 (C-1″), 104.0 (C-10), 115.3 (C-2′), 116.3 (C-5′), 121.2 (C-1′), 121.6 (C-6′), 133.3 (C-3), 144.8 (C-4′), 148.5 (C-3′), 156.5 (C-2), 156.7 (C-9), 161.2 (C-5), 164.1 (C-7), 177.4 (C-4). The data were consistent with the known compound.[18]

*Sakuranetin* (**5**), pale yellow crystal, mp151−153 °C (reported value 152−154 °C [19]); ESIMS: $m/z$ = 285 $[M-H]^-$; $^1$H NMR (DMSO-$d_6$) ppm: $\delta$ 2.73 (1H, dd, $J$ = 17.2, 2.8 Hz, H-3eq), 3.30 (1H, dd, $J$ = 17.2, 12.8 Hz, H-3ax), 3.87 (3H, s, 7-$OCH_3$), 5.48 (1H, dd, $J$ = 12.8, 2.8 Hz, H-2), 6.06 (1H, d, $J$ = 2.4 Hz, H-6), 6.09 (1H, d, $J$ = 2.4 Hz, H-8), 6.79 (2H, d, $J$ = 8.4 Hz, H-3′, 5′), 7.32 (2H, d, $J$ = 8.4 Hz, H-2′, 6′), 9.45 (1H, s, 4′−OH), 12.08 (1H, s, 5-OH). The data were consistent with the known compound.[20]

**High Performance Liquid Chromatography Analysis.** The caper fruits (400 g) from four growing areas were each extracted with boiling water (1600 mL × 2) for 2 h. The aqueous solution was condensed under reduced pressure to approximately 400 mL. Appropriate amount of 95% ethanol was then added to the condensed aqueous solution to make it 70% ethanol solution. This solution was left overnight and separated from precipitates by filtration. The filtrate was concentrated under reduced pressure and then subjected to 860021 macroporous absorption resin (Lu Kang Pharmaceutical, Shangdong, China) column (column size 4 L). The loaded sample was eluted with 30%, 50%, and 70% ethanol

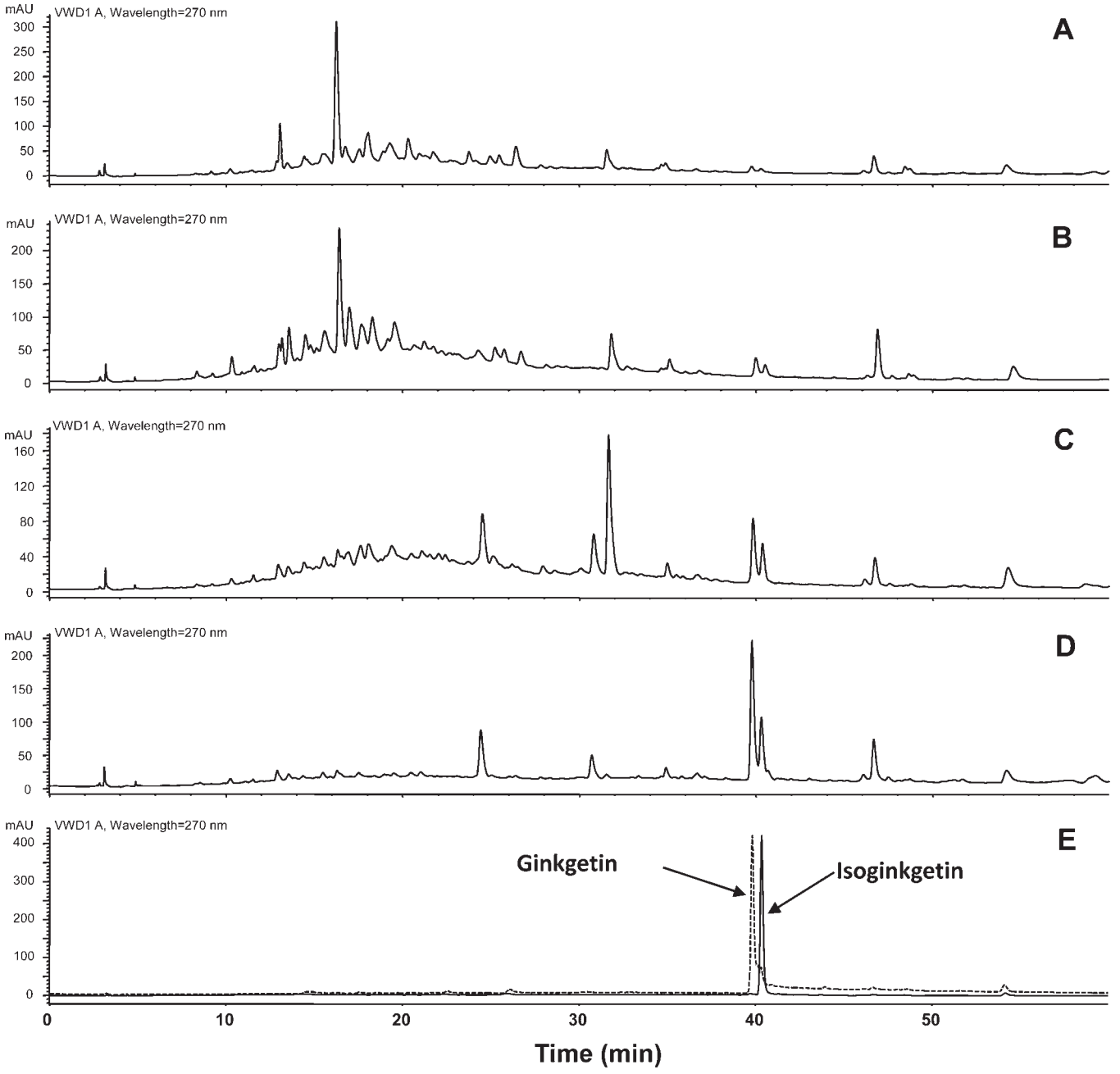


**Figure 2.** Representative HPLC chromatograms of isoginkgetin and ginkgetin in purified fractions of caper fruits collected from four major growing areas in northwest China (A, from Hetian 1; B, from Hami; C, from Hetian 2, D, from Yili, E, isoginkgetin and ginkgetin standards).

successively, with 10 L each. The 70% ethanol eluting fraction was subjected to polyamide column chromatography (column size 500 mL) eluted with 50% and 95% ethanol, respectively. The 95% ethanol eluate was dried under reduced pressure and reconstituted in 10 mL MeOH for HPLC analysis.

Pure compounds **1** and **2** obtained from above-mentioned procedure were used as standards. The separation was performed on Agilent 1100 HPLC system (Palo Alto, CA) detected at 270 nm with a flow rate of 1.0 mL/min. The column used was a 250 mm ×4.6 mm i.d., 5 $\mu$m, Diamonsil−C18 analytical column (Scarborough, Canada). Column oven temperature was set up at 25 °C. Mobile phases consisted of A (water containing 0.1% formic acid) and B (acetonitrile) with the following gradient: 0−10 min, from 5% to 25% B; 10−25 min, from 25% to 40% B; 25−35 min, from 40% to 60% B; 35−45 min, from 60% to 80% B; 45−60 min, 80% B. The contents of the two biflavonoids in dried caper fruits were estimated by calculating the yield derived from the purified fraction.

**SEAP Reporter Assay.** RAW-Blue cells (Invitrogen, San Diego, CA) are derived from RAW264.7 macrophages with chromosomal integration of a SEAP reporter construct inducible by NF-$\kappa$B and AP-1. RAW-Blue mouse macrophage cell lines were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% (v/v) fetal bovine serum (FBS) (Hyclone, Logan, UT) and zeocineosin (200 $\mu$g/mL). All cell culture reagents were purchased from Invitrogen (San Diego, CA). RAW-Blue cells ($1 \times 10^5$ cells/well) were pretreated with compounds for 3 h, followed by stimulation with LPS (100 ng/mL, Invitrogen, San Diego, CA) for 18 h. The supernatants were collected for SEAP secretion assay. QUANTI-Blue powder was

**Table 1. Concentrations of Ginkgetin and Isoginkgetin in Caper Fruits Collected from Four Major Growing Areas in Northwest China**

| | Hetian 1 | Hami | Hetian 2 | Yili |
|---|---|---|---|---|
| | | $\mu$g/g (dry weight) | | |
| ginkgetin | 0.21 | 0.86 | 3.22 | 24.18 |
| isoginkgetin | 0.09 | 0.46 | 1.70 | 8.30 |

dissolved in endotoxin-free water and sterile filtered (0.22 $\mu$m) (QuantiQuanta-blue substrate). RAW-Blue cell supernatant (40 $\mu$L/well) was added to QuantiQuanta-blue substrate (160 $\mu$L/well) and incubated at 37 °C for 0.5−1 h. Absorbance was measured at 620 nm in a PolarStar Microplate Reader (BMG Labtech, Durham, NC).

**Statistical Analysis.** The results for SEAP reporter assays were expressed as mean ± SD. Data were subjected to one-way ANOVA for statistical analyses by SigmaStat (version 3.5) and Student−Newman−Keuls Method was used for multiple comparison procedures. $P < 0.01$ is considered as significant difference.

## RESULTS AND DISCUSSION

**Characterization of Compounds.** Isolation of flavonoids was performed in the medium polar fractions of The caper fruit extract, which according to our previous report[6] are the most bioactive anti-inflammatory fractions. After repeated separation by various chromatographic methods, five flavonoids were obtained: isoginkgetin (**1**), ginkgetin (**2**), kaempferol-3-*O*-rutinoside (**3**), quercetin-3-*O*-rutinoside (**4**) and sakuranetin (**5**) (Figure 1). Their structures were elucidated by ESI-MS, $^1$H and $^{13}$C NMR spectra and compared with the literature. To our knowledge, two biflavonoids, isoginkgetin (**1**) and ginkgetin (**2**), as well as sakuranetin (**5**), were identified in caper for the first time. Remarkably, it is also the first time that biflavonoids have ever been found in Capparidaceae.

**HPLC Analysis.** Since the two biflavonoids isoginkgetin and ginkgetin had never been identified in caper and even in Capparidaceae before, HPLC analyses were conducted to verify and quantitate these two compounds in the caper fruits collected from major growing areas in the Xinjiang Uygur Autonomous Region of China. The concentrations of isoginkgetin (**1**) and ginkgetin (**2**) from certain samples were too low to be detected in crude extracts of caper fruits. Therefore, identification and quantitation were conducted in the purified fractions. The representative chromatograms of the two biflavonoids in four caper fruit samples were shown in Figure 2A−D. The concentrations of the two compounds in dried fruits were estimated based on calculating the yields of each purification step (Table 1). We realized that this calculation may underestimate the values due to losses during each purification step. Better quantitation in crude extracts using mass spectrometer currently under development in our lab. The extreme low abundances of two compounds may partly explain why they were not identified before. Moreover, the concentrations varied significantly among these four samples (Table 1), even for the two samples collected not too far away from each other (two locations within Hetian, Hetian 1 and Hetian 2). Fortunately, we happened to work on the plant materials containing high levels of the two biflavonoids, leading to the identification of these two compounds.

**SEAP Reporter Assay.** NF-$\kappa$B plays a central role in regulating the expression of inflammatory cytokines, chemokines, cell adhesion molecules, growth factors, and immuno-receptors.[21,22] Hence,

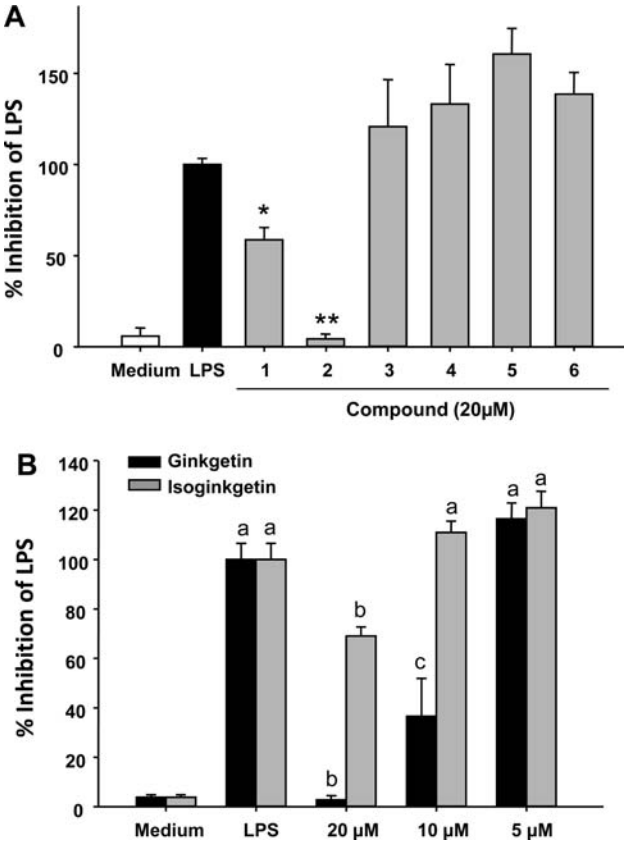


**Figure 3.** Results of SEAP reporter assay induced by LPS for compounds isolated from caper fruits, the concentration of individual compounds is 20 $\mu$M (A). Comparison was made between the data from LPS alone and LPS plus compounds treated cells (mean ± SD, $n$ = 3, *$P < 0.01$, **$P < 0.001$). The corresponding compound names for **1**−**6** are isoginkgetin, ginkgetin, kaempferol-3-*O*-rutinoside, quercetin-3-*O*-rutinoside, sakuranetin and thevetiaflavone, respectively. The results of dose−response experiments of SEAP reporter assay induced by LPS for isoginkgetin and ginkgetin from 20 to 5 $\mu$M (B) (mean ± SD, $n$ = 3). Comparison was made between the data from LPS alone and LPS plus compounds treated cells Means with different letters are different ($P < 0.01$).

NF-$\kappa$B represents attractive targets for developing anti-inflammatory therapeutics.[23,24] In this study, all five compounds (**1**−**5**) from this study and one compound (thevetiaflavone) reported in a previous study[6] were screened for their activities in inhibiting NF-$\kappa$B activation. Thevetiaflavone was included in this study because its role as a NF-$\kappa$B inhibitor is unknown. The result showed that only isoginkgetin (**1**) and ginkgetin (**2**) displayed inhibitory effects, and the effect of ginkgetin (**2**) was much greater than that of isoginkgetin (**1**) (Figure 3A). A dose−response experiment was further carried out for isoginkgetin (**1**) and ginkgetin (**2**) from 5 $\mu$M to 20 $\mu$M (Figure 3B). The $IC_{50}$ value of ginkgetin (**2**) was calculated at 7.5 $\mu$M, whereas isoginkgetin (**1**) failed to inhibit NF-$\kappa$B activation at 10 and 5 $\mu$M. This indicated that ginkgetin (**2**) is a strong NF-$\kappa$B inhibitor and warrants further investigation.

The biflavonoids isoginkgetin (**1**) and ginkgetin (**2**) were originally isolated from *Ginkgo biloba* L.[25] As major phytochemicals in *Ginkgo biloba* L., both isoginkgetin (**1**) and ginkgetin (**2**) have been implicated as having anti-inflammatory effects.[26] For instance, ginkgetin was previously found to be an inhibitor of $cPLA_2$.[27] Ginkgetin (**2**) was also reported to suppress COX-2 expression from lipopolysaccharide

(LPS)-treated macrophages without affecting COX-2 activity, while an in vivo study has also revealed that ginkgetin (**2**) inhibited COX-2 expression and $PGE_2$ production from mouse skin induced by 3-d treatment of 12-*O*-tetradecanoylphorbol-13-acetate (TPA).[28] While *Ginkgo biloba* extracts have been shown to inhibit activity of NF-$\kappa$B,[29,30] there are no studies thus far to demonstrate specific compounds in *Ginkgo biloba* extracts that inhibited NF-$\kappa$B activation. Therefore, results from this study may also suggest the need to isolate additional anti-inflammatory bioactive compounds previously not considered in *Gingko biloba*.

Structurally, ginkgetin (**2**) and isoginkgetin (**1**) are very similar with the only difference on the position of one methoxy group (Figure 1). We do not have good explanation at this point why and how this minor structural difference accounts for the observed difference in inhibitory bioactivity. Apparently, substitution at one of or both of the two positions is an important factor determining its effect. Moreover, the information regarding the bioavailability of ginkgetin (**2**) remains unknown and the mechanism of absorption in the gastrointestinal tract has yet to be determined. Caper is one of the most common aromatic plants found in the Mediterranean Basin and have been a part of Mediterranean cuisine for thousands of years. The fresh aerial parts, specially the pickled flower buds, unripe fruits, and shoots, are stored in salt, vinegar, or brine and used as an appetizer or as a complement to other foods.[1] A serving of capers (10 g) was estimated to provide 65 mg of flavonoid glycosides or the equivalent 40 mg of aglycone.[31] Therefore, the flavonoids ingested by normal use of caper in the diet could reach to a considerable amount and show bioactivity.

In conclusion, two biflavonoids were identified from caper fruits for the first time in the present study, of which ginkgetin (**2**) showed inhibitory effects in vitro in NF-$\kappa$B activation at low micromole levels. The in vivo effects and clinical implications of these findings have yet to be determined.

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