Quantitative analysis and anti-inflammatory effects of *Gleditsia sinensis* thorns in RAW 264.7 macrophages and HaCaT keratinocytes

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**Abstract.** *Gleditsia sinensis* thorns have traditionally been used to treat edema and carbuncles and drain abscesses. In the present study, a simultaneous analysis of four flavonoids [(+)-catechin, (-)-epicatechin, eriodictyol and quercetin] and two phenolic compounds (caffeic acid and ethyl gallate), obtained from a 70% ethanol extract of *G. sinensis*, was performed using high-performance liquid chromatography-photodiode array techniques. In addition, the inhibitory activities of the solvent fractions from a *G. sinensis* extract and its major constituents on the lipopolysaccharide-stimulated production of inflammatory mediators by macrophage RAW 264.7 cells and the tumor necrosis factor (TNF)-α and interferon (IFN)-γ (TI)-stimulated production of chemokines by HaCaT keratinocytes were investigated. The established analytical method showed high linearity, with a correlation coefficient of ≥0.9998. The limits of detection and quantification of the six compounds were 0.037-0.425 and 0.124-1.418 µg/ml, respectively. The ethyl acetate fraction inhibited nitric oxide and prostaglandin E₂ production in RAW 264.7 cells and the production of thymus- and activation-regulated chemokine (TARC) in HaCaT cells more than did the other fractions. Furthermore, the six compounds reduced the production of TARC, macrophage-derived chemokine and regulated upon activation normal T-cell expressed and secreted in TI-stimulated HaCaT cells; in particular, ethyl gallate and quercetin exhibited a significant dose-dependent inhibition. Further elucidation of the signaling pathways involved in the T-helper cell 2 chemokine inhibition by *G. sinensis* is necessary to facilitate the design of therapeutic agents for the inflammatory response.

**Introduction**

The inflammatory response is a complex reaction of the immune system that is regulated by numerous inflammatory mediators, such as nitric oxide (NO), cytokines/chemokines and prostaglandins (1). These mediators are known to contribute to the regulation of a wide array of physiological and pathological processes following an immune response and inflammation (1,2). The role of macrophages in mediating numerous different immunopathological phenomena during inflammation, including the overproduction of inflammatory cytokines and mediators, such as tumor necrosis factor-α (TNF-α), interleukin (IL)-1β, IL-6, NO and prostaglandin E₂ (PGE₂) is crucial. NO is synthesized by inducible NO synthase (iNOS), and PGE₂ is synthesized by cyclooxygenase-2 (COX-2) (3,4).

Inflammatory chemokines play a fundamental role in attracting a diverse set of effector leukocytes to sites of inflammation (5). Various chemokines have been shown to affect the immunopathology of inflammatory skin diseases. The thymus- and activation-regulated chemokine (TARC/CCL17) has been shown to be upregulated by keratinocytes in atopic dermatitis lesional skin (6); therefore, the modulation of TARC production in keratinocytes may contribute to the pathological processes of inflammatory skin diseases, such as atopic dermatitis. The serum level of the macrophage-derived chemokine (MDC/CCL22) has been reported to be significantly elevated in patients with atopic dermatitis, and increased levels of these chemokines were associated with increased disease severity (7). Furthermore, enhanced production of regulated upon activation normal T-cell expressed and secreted (RANTES/CCL5) has been found in inflammatory diseases of the skin, including atopic dermatitis and psoriasis (8).

The thorns of *Gleditsia sinensis* (Leguminosae) are widely used in traditional Chinese and Korean medicine for the treatment of several diseases, including tumor-related diseases, obesity and thrombosis (9). Previous studies have investigated the biological activity of the chemical constituents of...
G. sinensis thorns, such as stigmasterol (10), ellagic acid glycosides (11), flavonoids (12) and lupane acid (13); however, the anti-inflammatory effects of G. sinensis thorn extracts remain unclear. The aim of the present study, therefore, was to investigate the anti-inflammatory activities of the solvent fractions from a G. sinensis extract and its major constituents on the lipopolysaccharide (LPS)-stimulated production of inflammatory mediators by RAW 264.7 macrophages and the TNF-α and IFN-γ (TI)-stimulated production of chemokines by HaCaT keratinocytes.

Materials and methods

Plant material. Thorns of G. sinensis were purchased in October 2008 from Omniherb (Yeongcheon, Republic of Korea). The origin of these materials was confirmed taxonomically by Professors Je-Hyun Lee (Dongkuk University, Gyeonggi, Republic of Korea) and Young-Bae Seo (Daejeon University, Daejeon, Republic of Korea). A voucher specimen (2008-ST22) has been deposited at the K-herb Research Center, Korea Institute of Oriental Medicine (Daejeon, Republic of Korea).

Extraction and solvent fractionation. The dried thorns of G. sinensis (10 kg) were extracted three times with 70% ethanol by sonication for 1 h. The extracted solution was filtered through filter paper and evaporated to dryness (450 g). The ethanol extract was suspended in water (1 liter) and then divided into four fractions, such as 70% ethanol, n-hexane, ethyl acetate and n-butanol (1.5 liters each, three times) to give extracts of 33.8, 103.2 and 119.1 g, respectively.

Chemicals and reagents. (+)-Epicatechin, ethyl gallate and quercetin (all with purity ≥99.0%) were purchased from Extrasynthese (Cayman Chemical Co., Ann Arbor, MI, USA) and NO (Griess Reagent System; Promega Co., Madison, WI, USA), according to the manufacturers’ instructions. RAW 264.7 (3x10^6 cells/well) and HaCaT (1x10^5 cells/well) cells were incubated in 96-well plates with various concentrations of the test materials for 24 h. Following the addition of CCK-8 reagent to each well the cells were further incubated for 4 h. A Benchmark Plus microplate reader (Bio-Rad, Hercules, CA, USA) was used to measure absorbance at 450 nm, and the cell viability percentage was calculated using the following formula: Cell viability (%) = [(mean absorbance in test wells - mean absorbance in blank wells) / mean absorbance in control wells - mean absorbance in blank wells] × 100.

Cytotoxicity assay. Cell viability was assessed using the Cell Counting Kit-8 (CCK-8) reagent (Dojindo Laboratories, Kumamoto, Japan), according to the manufacturer’s instructions. RAW 264.7 (3x10^6 cells/well) and HaCaT (1x10^5 cells/well) cells were incubated in 96-well plates with various concentrations of the test materials for 24 h. Following the addition of CCK-8 reagent to each well the cells were further incubated for 4 h. A Benchmark Plus microplate reader (Bio-Rad, Hercules, CA, USA) was used to measure absorbance at 450 nm, and the cell viability percentage was calculated using the following formula: Cell viability (%) = [(mean absorbance in test wells - mean absorbance in blank wells) / mean absorbance in control wells - mean absorbance in blank wells] × 100.

Measurement of PGE_2 and NO production. RAW 264.7 cells were plated in 48-well plates at a density of 2.5x10^5 cells/well and incubated overnight. The cells were then treated with LPS (1 µg/ml; Sigma-Aldrich, St. Louis, MO, USA) in the presence or absence of various concentrations of the test materials. Following incubation for 24 h, ELISA kits were used to analyze the supernatants for the levels of PGE_2 (Cayman Chemical Co., Ann Arbor, MI, USA) and NO (Griess Reagent System; Promega Biotech Co., Ltd., Madison, WI, USA), according to the manufacturers’ instructions. N(G)-Monomethyl-L-arginine (L-NMMA; Sigma-Aldrich) and indomethacin (Sigma-Aldrich) were used as positive controls to inhibit the production of NO and PGE_2, respectively.

Measurement of chemokine production. HaCaT cells (1x10^6 cells/well) were cultured in six-well plates in medium
containing 10% fetal bovine serum. After reaching confluence, the cells were washed and treated with the test materials in 1 ml serum-free medium containing Ti (10 ng/ml TNF-α and 10 ng/ml IFN-γ; R&D Systems Inc., Minneapolis, MN, USA) for 24 h. The supernatants of the cells were harvested, and the production of TARC, MDC and RANTES was quantified using ELISA (R&D Systems, Inc.), which was performed according to the manufacturer's instructions.

Statistical analysis. All experiments of the present study were performed at least in triplicate. Significant differences between the treatment groups were identified using one-way analysis of variance and multigroup comparisons were performed using the Dunnett's test. P<0.05 was considered to indicate a statistically significant difference.

Results

Calibration curves, LOD and LOQ. The linearity of the peak area (y) versus concentration (x, µg/ml) curve for each component was used to calculate the contents of the six constituents in each fraction of the G. sinensis extract. The correlation coefficients (r²) of the calibration curves of the six constituents were ≥0.9998. The line equations and r² of the calibration curves are shown in Table I. The ranges of LOD and LOQ were 0.037-0.425 and 0.124-1.418 µg/ml, respectively (Table I).

HPLC analysis. Good separation chromatograms were obtained using mobile phases consisting of 1.0% (v/v) aqueous acetic acid (solvent A) and acetonitrile with 1.0% (v/v) acetic acid (solvent B). Quantification was achieved using PDA detection at 280 nm, based on retention times and ultraviolet spectra. Fig. 1 shows the representative HPLC chromatogram of the standards and each fraction of the G. sinensis extract, with detection of eluents at 280 nm. Reproducibility was assessed by measuring repeatedly the retention times and peak areas of six independently prepared samples of analytes. The reproducibility of the six compounds ([+]-catechin, caffeic acid, (-)-epicatechin, eriodictyol, ethyl gallate and quercetin) was less than the relative standard deviation (RSD) 1.5% for peak responses and less than the RSD 0.2% for retention times (data not shown). The contents of the six constituents are summarized in Table II.

Table I. Linearities, correlation coefficients, LODs and LOQs of the six constituents (n=3).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Linear range (µg/ml)</th>
<th>Regression equation</th>
<th>Correlation coefficient (r²)</th>
<th>LOD (µg/ml)</th>
<th>LOQ (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(+)-Catechin</td>
<td>0.78-50.00</td>
<td>y=2,779.71x+274.75</td>
<td>0.9999</td>
<td>0.425</td>
<td>1.418</td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>0.78-50.00</td>
<td>y=34,405.46x-9,908.57</td>
<td>0.9998</td>
<td>0.039</td>
<td>0.132</td>
</tr>
<tr>
<td>(-)-Epicatechin</td>
<td>0.78-50.00</td>
<td>y=7,859.71x+1,909.87</td>
<td>0.9999</td>
<td>0.130</td>
<td>0.433</td>
</tr>
<tr>
<td>Ethyl gallate</td>
<td>0.78-50.00</td>
<td>y=24,543.65x+2,271.17</td>
<td>0.9999</td>
<td>0.051</td>
<td>0.171</td>
</tr>
<tr>
<td>Eriodictyol</td>
<td>0.78-50.00</td>
<td>y=38,836x+6,410.25</td>
<td>0.9999</td>
<td>0.037</td>
<td>0.124</td>
</tr>
<tr>
<td>Quercetin</td>
<td>0.78-50.00</td>
<td>y=13,439.20x-676.53</td>
<td>0.9998</td>
<td>0.118</td>
<td>0.395</td>
</tr>
</tbody>
</table>

'y', peak area (mAU) of constituents; x, concentration (µg/ml) of constituents. LOD = 3 x signal-to-noise ratio. LOQ = 10 x signal-to-noise ratio. LOD, limit of detection; LOQ, limit of quantification.

G. sinensis extract inhibits NO and PGE₂ production in RAW 264.7 cells and TARC production in HaCaT cells. The cytotoxic effect of the G. sinensis extract on RAW 264.7 and HaCaT cells was measured first. The cells were exposed to various concentrations (2-200 µg/ml) of the G. sinensis extract for 24 h. The non-toxic concentrations of the test materials were used in subsequent experiments (data not shown). To determine the effects of the G. sinensis extract on NO and PGE₂ production in LPS-stimulated RAW 264.7 cells, the cells were treated with different concentrations of the G. sinensis extract (25, 50 and 100 µg/ml) and then stimulated by LPS (1 µg/ml) for 24 h. The G. sinensis extract suppressed LPS-stimulated NO production in a dose-dependent manner (Fig. 2A). LPS greatly stimulated NO production (5.84±0.220 nM) in RAW 264.7 cells, whereas the G. sinensis extract significantly decreased NO production [1.872±0.513 µM (P<0.01) at a dose of 25 µg/ml, 0.974±0.128 µM (P<0.01) at a dose of 50 µg/ml and 0.205±0.020 µM (P<0.01) at a dose of 100 µg/ml] compared with that observed in LPS-stimulated RAW 264.7 cells. Furthermore, LPS-stimulated RAW 264.7 cells exhibited increased PGE₂ production compared with the controls, whereas the G. sinensis extract decreased the production of PGE₂ relative to the production observed in the LPS-stimulated RAW 264.7 cells (Fig. 2B). The effects of the G. sinensis extract on TARC production in TI-stimulated HaCaT cells were assessed by treating the cells with different concentrations of the G. sinensis extract (50, 100 and 200 µg/ml) and then initiating TI stimulation for 24 h. The G. sinensis extract suppressed TI-stimulated TARC production in a dose-dependent manner (Fig. 2C). TI-treated cells showed significantly decreased TARC production compared with the controls, whereas the G. sinensis extract inhibited the production of TARC in HaCaT cells treated with TI. NO production is the hallmark of the activation of macrophages. In the present experiment, NO production (4.308±0.225 µM) was
observed after 24 h of LPS incubation (1 µg/ml). As shown in Fig. 3A, treatment with the n-hexane, ethyl acetate and n-butanol fractions suppressed the NO production. In addition, the n-hexane and ethyl acetate fractions decreased PGE$_2$ production compared with that observed in LPS-stimulated RAW 264.7 cells (P<0.01, Fig. 3B). TARC production was increased 2.7-fold in the TI-treated cells (28.30±0.820 ng/ml) compared with that in the control cells (10.16±1.540 ng/ml) (P<0.01), whereas cells treated with the ethyl acetate fraction showed the most significant reductions in TARC production compared with the TI-treated cells (P<0.01, Fig. 3C). These results showed that the biological effects of G. sinensis were maximized by the ethyl acetate fraction.

Major constituents of the G. sinensis extract inhibit NO and PGE$_2$ production in RAW 264.7 cells and chemokine production in HaCaT cells. To assess the effects of the major constituents of the G. sinensis extract on LPS-stimulated NO and PGE$_2$ production in RAW 264.7 macrophages, cells were treated with various concentrations of these major constituents and 1 µg/ml LPS for 24 h. LPS stimulation caused a marked accumulation of proinflammatory mediators (NO and PGE$_2$) in the culture medium. Of note, all major constituents of the G. sinensis extract significantly reduced the LPS-stimulated production of NO (Fig. 4A) and PGE$_2$ (Fig. 4B) in a dose-dependent manner. Treatment of HaCaT cells with TI for 24 h led to a 1.9-fold increase in TARC levels (6.85±0.51 ng/ml) compared with that observed in the vehicle-treated control group (3.52±0.29 ng/ml) (P<0.01); however, TARC production was significantly inhibited in a dose-dependent manner in the ethyl gallate-, eriodictyol- and quercetin-treated cells (P<0.01) (Fig. 4C). In addition, MDC production was increased in the TI-treated cells (264.80±12.76 ng/ml) compared with that in the vehicle-treated control group (P<0.01), and its levels were significantly reduced following ethyl gallate and quercetin treatment (P<0.01) (Fig. 4D). TI-treated cells exhibited a significantly increased production of RANTES.
Table II. Contents of six constituents of *G. sinensis* (n=3).

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Caffeic acid</th>
<th>(+)-Catechin</th>
<th>(-)-Epicatechin</th>
<th>Ethyl gallate</th>
<th>Eriodictyol</th>
<th>Quercetin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean SD</td>
<td>Mean SD RSD (%)</td>
<td>Mean SD RSD (%)</td>
<td>Mean SD RSD (%)</td>
<td>Mean SD RSD (%)</td>
<td>Mean SD RSD (%)</td>
</tr>
<tr>
<td>70% EtOH</td>
<td>8.63 0.18 2.07</td>
<td>2.79 0.04 1.44</td>
<td>4.92 0.03 0.55</td>
<td>2.89 0.06 2.04</td>
<td>0.29 0.01 3.55</td>
<td>2.54 0.06 2.21</td>
</tr>
<tr>
<td>n-Hexane</td>
<td>N.D. - - 0.18</td>
<td>0.18 0.00 1.18</td>
<td>6.07 0.02 1.45</td>
<td>5.33 0.08 1.45</td>
<td>1.26 0.04 1.50</td>
<td>4.98 0.05 1.30</td>
</tr>
<tr>
<td>EtOAc</td>
<td>5.33 0.08 1.45</td>
<td>1.04 0.01 3.14</td>
<td>5.61 0.06 0.99</td>
<td>5.28 0.08 1.54</td>
<td>5.61 0.06 0.99</td>
<td>5.61 0.06 1.54</td>
</tr>
<tr>
<td>n-BuOH</td>
<td>3.78 0.06 1.54</td>
<td>0.28 0.00 0.55</td>
<td>1.18 0.03 1.56</td>
<td>3.78 0.06 1.54</td>
<td>0.28 0.00 0.55</td>
<td>1.18 0.03 1.56</td>
</tr>
<tr>
<td>Water</td>
<td>3.78 0.06 1.54</td>
<td>0.28 0.00 0.55</td>
<td>1.18 0.03 1.56</td>
<td>3.78 0.06 1.54</td>
<td>0.28 0.00 0.55</td>
<td>1.18 0.03 1.56</td>
</tr>
</tbody>
</table>

SD, standard deviation; RSD, relative standard deviation; EtOH, ethanol; EtOAc, ethyl acetate; n-BuOH, n-butanol; N.D., not detected.

Figure 2. Effects of the extract of *Gleditsia sinensis* on the LPS-stimulated production of NO and PGE<sub>2</sub> in RAW 264.7 cells and the TI-stimulated production of TARC in HaCaT cells. The production of (A) NO and (B) PGE<sub>2</sub> was assayed in the culture medium of cells treated with extracts of *G. sinensis* (25, 50, and 100 µg/ml) and then with LPS (1 µg/ml) for 24 h. L-NMMA (25 µM) was used as a positive control drug. (C) The production of TARC was assayed in the culture medium of cells treated with extracts of *G. sinensis* (50, 100, and 200 µg/ml), followed by TI (TNF-α, 10 ng/ml; IFN-γ, 10 ng/ml) for 24 h. Silymarin (25 µg/ml) was used as a positive control drug. Each bar represents the mean values obtained from three independent experiments. **P<0.01 vs. the vehicle control group; ***P<0.001 vs. LPS/TI-treated cells. LPS, lipopolysaccharide; NO, nitric oxide; PGE<sub>2</sub>, prostaglandin E<sub>2</sub>; TARC, thymus- and activation-regulated chemokine; TNF-α, tumor necrosis factor-α; IFN-γ, interferon-γ; TI, TNF-α and IFN-γ; L-NMMA, N(G)-monomethyl-L-arginine.
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![Image](image_url)

Figure 3. Effects of the fractions of Gleditsia sinensis extract on the LPS-stimulated production of NO and PGE_2 in RAW 264.7 cells and the TI-stimulated production of TARC in HaCaT cells. The production of (A) NO and (B) PGE_2 was assayed in the culture medium of cells treated with solvent fractions and then stimulated with LPS (1 µg/ml) for 24 h. L-NMMA (25 µM) was used as a positive control drug. (C) The production of TARC was assayed in the culture medium of cells treated with solvent fractions and then stimulated with TI (TNF-α, 10 ng/ml; IFN-γ, 10 ng/ml) for 24 h. Silymarin (25 µg/ml) was used as a positive control drug. Each bar represents the mean values obtained from three independent experiments. **P<0.01 vs. the vehicle control group; *P<0.05 and ***P<0.01 vs. the LPS/TI-treated cells. LPS, lipopolysaccharide; NO, nitric oxide; PGE_2, prostaglandin E_2, TARC, thymus- and activation-regulated chemokine; TNF-α, tumor necrosis factor-α; IFN-γ, interferon-γ; TI, TNF-α and IFN-γ; L-NMMA, N(G) -monomethyl-L-arginine.

(4,652.93±66.95 ng/ml), relative to that observed in the control cells (P<0.01). This increase was inhibited dose-dependently by ethyl gallate (2,530.11±8.67 ng/ml at 50 µg/ml, P<0.01; 1,068.30±95.01 ng/ml at 100 µg/ml, P<0.01), eriodictyol (3,987.98±127.92 ng/ml at 50 µg/ml, P<0.05; 2,433.12±184.17 ng/ml at 100 µg/ml, P<0.01) and quercetin (2,728.46±119.93 ng/ml at 50 µg/ml, P<0.01; 414.34±27.59 ng/ml at 100 µg/ml, P<0.01) (Fig. 4E). Among these compounds, ethyl gallate and quercetin, which were most abundant both in the G. sinensis extract and in each solvent fraction (particularly in the ethyl acetate fraction) significantly inhibited the RANTES, MDC and TARC expression in HaCaT cells in a dose-dependent manner, whereas the same expression was not significantly reduced by other constituents in TI-treated cells.

Discussion

The seeds and fruits of G. sinensis are widely used in Chinese, Japanese and Korean herbal medicine for the treatment of numerous diseases, such as apoplexy, headache, asthma and scabies (14). Despite the fact that the thorns of G. sinensis have been used for the prevention, as well as the treatment, of carbuncles, scabies and other inflammatory diseases (15), the anti-inflammatory effect has not yet been fully elucidated. In the present study, the concentrations of the chemical constituents of G. sinensis and their anti-inflammatory effects in G. sinensis extracts, based on different solvent fractions and single compounds, were investigated.

Two phenolic compounds (caffeic acid and ethyl gallate) and four flavonoids [(+)-catechin, (-)-epicatechin, eriodictyol...
Figure 4. Effects of the major constituents of the *Gleditsia sinensis* extract on the LPS-stimulated production of NO and PGE$_2$ in RAW 264.7 cells and the TI-stimulated production of chemokines in HaCaT cells. The production of (A) NO and (B) PGE$_2$ was assayed in the culture medium of cells treated with the major constituents of the *G. sinensis* extract and then stimulated with LPS (1 µg/ml) for 24 h. L-NMMA (25 µM) was used as a positive control drug. (C) The production of TARC was assayed in the culture medium of cells treated with the major constituents of the *G. sinensis* extract and then stimulated with TI (TNF-α, 10 ng/ml; IFN-γ, 10 ng/ml) for 24 h. Silymarin (25 µg/ml) was used as positive control drug. Each bar represents the mean of three independent experiments. **P<0.01 vs. the vehicle control group; *P<0.05 and **P<0.01 vs. the LPS/TI-treated cells. LPS, lipopolysaccharide; NO, nitric oxide; PGE$_2$, prostaglandin E$_2$, TARC, thymus- and activation-regulated chemokine; TNF-α, tumor necrosis factor-α; IFN-γ, interferon-γ; TI, TNF-α and IFN-γ; L-NMMA, N(G)-monomethyl-L-arginine.
and quercetin) were analyzed in 70% ethanol extracts and solvent fractions (n-hexane, ethyl acetate, n-butanol and water) of *G. sinensis*; however, the constituents responsible for peaks at the retention times of 19.8, 20.7 and 22.5 min in the 70% ethanol extract and ethyl acetate fraction were not able to be identified. It is imperative that these constituents are identified and quantitatively determined in order for a correlation between the quantities of the major constituents of *G. sinensis* and the biological effects of this herbal medicine to be obtained.

The production of NO and PGE$_2$, two major mediators of inflammation, plays an important role in the immune response to numerous inflammatory stimuli. The free radical NO is produced by iNOS, and its overproduction has been associated with the pathology of an array of inflammatory disorders, such as septic shock (16). PGE$_2$, which is generated from arachidonic acid by COX-2, is produced in response to inflammatory stimuli, and abundant COX-2 expression promotes the proinflammatory signaling cascade (17). Thus, potential inhibitors of iNOS and COX-2 have been considered as possible anti-inflammatory agents. It was shown that the extraction solvent and the major constituents in *G. sinensis* reduced the levels of NO and PGE$_2$ in LPS-stimulated RAW 264.7 cells in a dose-dependent manner.

Exposure of keratinocytes to TI results in an abnormal expression of chemokines and cytokines, which is believed to increase the infiltration of monocytes/T cells into the site of inflammation (18,19). Chemokines are a superfamily of small cytokines that regulate the trafficking of various types of leukocytes (20). TARC/CCL17 is a member of the CC chemokine subfamily that is produced by various cell types, including keratinocytes, and is constitutively expressed in the thymus (21). MDC/CCL22 is closely associated with TARC and is constitutively produced by dendritic cells, B
cells, keratinocytes, epithelial cells and macrophages (22). RANTES/CCL5 belongs to the CC chemokine family, the members of which recruit and activate different subtypes of leukocytes, such as T cells, eosinophils, basophils, monocytes or mast cells (23). The effect of the extract and solvent fractions of *G. sinensis* on TARC production in T1-stimulated HaCaT cells was examined and it was shown that the major constituents of *G. sinensis* reduced the production of TARC, MDC and RANTES in T1-stimulated HaCaT cells; in particular, ethyl gallate and quercetin yielded a significant dose-dependent inhibition.

In conclusion, the *G. sinensis* extract was successively partitioned to identify the fraction that contained the major constituent compounds. It was found that all the reference compounds were present in the ethyl acetate fraction and that larger amounts were present in this fraction than in any other fraction. Furthermore, the ethyl acetate fraction decreased the levels of NO and PGE$_2$ in RAW264.7 cells and the expression of TARC, MDC and RANTES in HaCaT cells to a greater extent than the other fractions. Further elucidation of the signaling pathways involved in T-helper cell 2 chemokine inhibition by *G. sinensis* is required for the facilitation of the design of therapeutic agents for the inflammatory reaction.

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**References**


