An *in vitro* inhibitory effect on RAW 264.7 cells by anti-inflammatory compounds from *Smilax corbularia* Kunth

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**Summary**

*Background:* *Smilax corbularia* is a Thai medicinal plant locally known as ‘Hua-Khao-Yen Neua’, which is used for treating inflammatory conditions.

*Objective:* To evaluate the anti-inflammatory effect of *S. corbularia* extracts and its isolated compounds by determination of inhibitory effects on lipopolysaccharide-stimulated PGE$_2$ release, and TNF-α and NO production from RAW 264.7 cells.

*Methods:* The inhibitory effect of aqueous and ethanolic extracts of this plant were determined on LPS-induced NO production, TNF-α and PGE$_2$ release in RAW 264.7 cells, as an *in vitro* indication of possible anti-inflammatory activity. The compounds from active extract were isolated by bioassay-guided fractionation.

*Results:* Only the ethanolic extract of this plant inhibited TNF-α and NO production, with IC$_{50}$ values of 61.97, and 83.90 µg/ml respectively. Three flavonols, engeletin, astilbin and quercetin were isolated from the ethanolic extract. Quercetin possessed the highest inhibitory effect on NO production with IC$_{50}$ values of 11.2 µg/ml (37.1 µM), whereas engeletin and astilbin had no activity (IC$_{50}$ >100 µg/ml). All three flavonols possessed potent inhibition of PGE$_2$ release with IC$_{50}$ values of 14.4, 19.6 and 19.9 µg/ml (33.2, 43.5 and 65.8 µM) respectively. Quercetin also exhibited the highest inhibitory effect on TNF-α production (IC$_{50}$ = 1.25 µg/ml or 4.14 µM), but engeletin and astilbin had no activity.

*Conclusion:* This is the first report of isolated compounds from *S. corbularia* with potential anti-inflammatory effects, and the results support the use of this plant by Thai traditional doctors for treatment of inflammatory diseases. (Asian Pac J Allergy Immunol 2012;30:268-74)

**Key words:** Smilax corbularia Kunth, Anti-inflammatory, PGE$_2$, TNF-α, Nitric oxide, RAW 264.7

**Introduction**

*Smilax corbularia* Kunth (Smilacaceae) is a Thai medicinal plant called ‘Hua-Khao-Yen-Neua’ in Thai. It has long been used as a common ingredient in many traditional Thai preparations for treatment of dermatopathy, lymphopathy, inflammation, cancers, venereal diseases, and leprosy.$^1$ From selective interviews with 23 folk doctors from all parts of Thailand, it was found that this plant was used for anti-inflammatory conditions such as arthritis, cancer, postpartum pain reduction and to increase immunity of HIV patients.$^2$ There are only two previous studies on the anti-inflammatory effects of this species. Oral administration of the ethanolic extract of *S. corbularia* rhizomes (1600 mg/kg) significantly suppressed the paw edema induced by carrageenan in rats.$^3$ The ethanolic and water extracts of this plant were examined for inhibitory activities against lipopolysaccharide (LPS)-induced nitric oxide (NO) production in RAW 264.7 cell lines, exhibiting mild inhibitory activity with IC$_{50}$ values of 61.2 and 61.0 µg/ml.$^4$ Little is known about the phytochemistry of this species apart from a report of isolated compounds from *S. corbularia* which were tested for their estrogenic effect.$^5$ Surprisingly, no further work has
been carried out on the mechanism of action for extracts and isolated compounds, in particular any inhibitory effect on lipopolysaccharide (LPS)-stimulated PGE\textsubscript{2} and TNF-\textalpha release and NO production by RAW 264.7 cells. Thus, this study aimed to evaluate the activity, relevant to any anti-inflammatory effect, of \textit{Smilax corbularia} Kunth extracts, by determination of inhibitory effects on LPS-stimulated prostaglandin E\textsubscript{2} (PGE\textsubscript{2}) release or COX-II inhibition, TNF-\textalpha and NO production by RAW 264.7 cells. The chemical basis of any observed effect would be determined by bioassay-guided fractionation and isolation of active compounds.

**Methods**

**Plant material**

The rhizomes of \textit{Smilax corbularia} Kunth (Smilacaceae) (HuaxKhao-Yen-Neua) were collected from Amphor Mae Taeng, Chiang Mai Province, Thailand. Authentication of plant material was carried out at the herbarium of the Department of Forestry, Bangkok, Thailand, where the herbarium voucher has been kept. A duplicate set has been deposited in the herbarium of Southern Center of Thai Medicinal Plants at the Faculty of Pharmaceutical Sciences, Prince of Songkla University, Hat-Yai, Songkhla, Thailand. They were cultured in RPMI-1640 medium supplemented with 0.1% sodium bicarbonate and 2 mM glucose, penicillin (100 \, \mu\text{g/ml}), streptomycin (100 \, \mu\text{g/ml}) and 10% FBS. The cells were harvested with trypsin-EDTA and diluted to a suspension in fresh medium before plating for experiments, as detailed below.

**Preparation of plant extracts**

The rhizomes of \textit{Smilax corbularia} were dried at 50\,\degree C, powdered and extracted by methods corresponding to those practiced by traditional doctors. The water extract was obtained by boiling dried plant material (100 g) for 30 min in 300 ml of distilled water, filtering and then freeze-drying the filtrate. For the ethanolic extracts, the plant material (100 g) was macerated with ethanol for 72 hours, then filtered and concentrated to dryness under reduced pressure. The water extract was dissolved in sterile water which was filtration sterilised (0.2 \, \mu\text{m}) and the ethanolic extract was dissolved in dimethyl sulfoxide (DMSO) to form stock solutions of 10 mg/ml which was used for serial dilutions for testing.

**Anti-inflammatory assay**

**Reagents**

Lipopoly saccharide (LPS, from \textit{Salmonella enteritidis}), 3-(4,5-dimethyl-2-thiazolyl)-2,5 diphenyl-2H-tetrazolium bromide (MTT) and l-nitroarginine (L-NA) were purchased from Sigma. RPMI-1640 medium, fetal bovine serum (FBS), penicillin-streptomycin and phosphate-buffer saline (PBS) were purchased from Biochrome. 96-well sterile microplates were purchased from Costar. Prostaglandin E\textsubscript{2} EIA Kit Monoclonal were purchased from Cayman Chemical Company and used according to the manufacturer’s instructions. Mouse TNF-\textalpha Quantikine ELISA was purchased from R&D Systems.

The RAW 264.7 cells were kindly provided by Assoc. Prof. Dr. Supinya Tewtrakul, Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Science, Prince of Songkla University, Hat-Yai, Songkhla, Thailand. They were cultured in RPMI-1640 medium supplemented with 0.1% sodium bicarbonate and 2 mM glucose, penicillin (100 \, \mu\text{g/ml}), streptomycin (100 \, \mu\text{g/ml}) and 10% FBS. The cells were harvested with trypsin-EDTA and diluted to a suspension in fresh medium before plating for experiments, as detailed below.

**Effects on LPS-induced NO release using RAW 264.7 cells**

The effect of extracts on NO production by murine macrophage-like RAW 264.7 cell lines was determined using a method modified from that previously reported.\textsuperscript{16} Briefly, the cells were seeded in 96-well plates with $1 \times 10^5$ cells/well and allowed to adhere for 1 h. After that the medium was replaced with fresh medium containing 5 \, \mu\text{g/ml} of LPS, together with test samples at various concentrations, and incubated for 24 h. NO production was determined by measuring the accumulation of nitrite in the culture supernatant using the Griess reagent. Cytotoxicity was also determined using the 3-(4,5-dimethyl-2-thiazolyl) 2,5-diphenyl-2H-tetrazolium bromide (MTT) colourimetric method. Briefly, after 24 h incubation with test samples, MTT solution (10 \, \mu\text{l}, 5 mg/ml in PBS) was added to the wells. After 4 h incubation, the medium was removed, and iso-propanol containing 0.04 M HCl was then added to dissolve the formazan produced by the cells. The optical density of formazan solution was measured with a microplate reader at 570 nm. The test compounds or extract were considered to be cytotoxic when the optical density of the sample-treated group was less than 80% of that in the control (vehicle-treated) group. Indomethacin was used as a positive control. The stock solution of each test sample was dissolved in DMSO, and the solution was added to the medium RPMI-1640 (final DMSO is not more than
0.2%). Percentage inhibition was calculated using the following equation and IC\textsubscript{50} values were determined graphically (n = 4):

\[
\text{Inhibition (\%)} = \frac{(A-B)\times100}{(A-C)}
\]

A − C: NO\textsubscript{2} − concentration (μM)

[A: LPS (+), sample (−); B: LPS (+), sample (+); C: LPS (−), sample (−)].

**Effects on LPS-stimulated PGE\textsubscript{2} release from RAW 264.7 cells**

The effect of extracts on LPS-stimulated PGE\textsubscript{2} production by the murine macrophage-like RAW 264.7 cell line was evaluated using a method modified from that previously reported. Briefly, the RAW 264.7 cells were seeded in 24-well plates (2.5 × 10\textsuperscript{5} cells/ml), and stimulated with 1 μg/ml lipopolysaccharide (LPS) in medium for 24 h. After the incubation period, the culture supernatants were collected, and the amount of PGE\textsubscript{2} was determined graphically (n = 4):

\[
\text{Inhibition (\%)} = \frac{(A\times B)}{(A – C)} \times 100
\]

A − C: LPS (−), sample (−)

[A: LPS (+), sample (−); B: LPS (+), sample (+); C: LPS (−), sample (−)].

**Statistical analysis**

The results are expressed as the mean ± SEM of three determinations. The IC\textsubscript{50} was calculated using the Prism programme (Graphpad, San Diego, California).

**Isolation of compounds from Smilax corbularia extract**

The dried powdered rhizomes of *Smilax corbularia* (1 kg) were macerated with ethanol, and the extract concentrated under reduced pressure to obtain 110 g of ethanolic extract (percentage of yield was 11%). The crude ethanolic extract (5 g) was then chromatographed over silica gel using 7:3 chloroform:methanol to give 10 fractions. Fraction 5, which contained the main compounds and had only three clear spots of compounds in TLC, was concentrated and gave crystals by methanol (721.2 mg). These crystals were redissolved and rechromatographed over a silica gel column using 8.5:1.5 chloroform:methanol. Ten millilitres of each fraction was collected. Compound 1 was obtained as a yellow brown powder (26.3 g, 0.53%, w/w) from fractions 27-30, and compound 2 as white crystals (49 mg, 0.98%, w/w) from fractions 34-37. Fraction 6 was rechromatographed over a silica gel column using 9:1 chloroform:methanol as final eluant, and 10 ml per fraction was collected; fractions 20-25, which showed one compound on TLC, were collected and washed with methanol to yield yellow crystals of compound 3 (12.6 mg, 0.25%, w/w).

**Structure elucidation**

The structures of the isolated compounds were determined by their NMR data [\textsuperscript{1}H and \textsuperscript{13}C on a Varian Unity Inova 500 spectrometer (500 MHz for \textsuperscript{1}H; 125 MHz for \textsuperscript{13}C)], UV spectra [Specord S100 spectrometer (Analytik Jena)], IR spectra [Perkin Elmer FTS FT-IR spectrometer], and EI mass spectra, both HRMS and LRMS, were obtained from a Thermo Finnigan MAT 95XL mass spectrometer. Optical rotations were obtained from a Perkin Elmer 341 spectrometer.

**Results**

**Isolated pure compounds from the ethanolic extract of Smilax corbularia**

**Compound 1** (Engeletin): C\textsubscript{21}H\textsubscript{22}O\textsubscript{10} (26.30 mg, 0.53% w/w) was a yellow brown powder; it showed HR-REIMS m/z [M]+ 434.1204 (Calc. for C\textsubscript{21}H\textsubscript{22}O\textsubscript{10} 434.1204), specific optical rotation [\(\alpha\)D] = +11.50 (c 0.20, MeOH), UV (MeOH) λ\textsubscript{max} (logε) 329.80 (4.76), 292.50 (5.30), 217.92 (5.46) nm, IR (KBr disc) λ\textsubscript{max} 3369.91, 2922.50, 1638.73, 1587.09, 1520.29, 1481.65, 1333.09, 1085.36, 778.04 cm\textsuperscript{-1}. The \textsuperscript{1}H-NMR (500 MHz in CDCl\textsubscript{3}) and \textsuperscript{13}C-NMR (125 MHz in CDCl\textsubscript{3}) spectra were identical to those reported for engeletin.\textsuperscript{9}

**Compound 2** (Astilbin): C\textsubscript{21}H\textsubscript{22}O\textsubscript{11} (49.00 mg, 0.98% w/w) was a white crystalline solid and was the major compound, it showed EI-MS m/z 450.1167 (Calc. for C\textsubscript{21}H\textsubscript{22}O\textsubscript{11} 450.1167), specific optical rotation [\(\alpha\)D] = -2.92 (c 0.25, MeOH), UV (MeOH) λ\textsubscript{max} (logε) 330.44 (4.41), 290.73 (4.99), 226.60 (5.02) and 214.94 (5.11) nm. IR (KBr disc)
λ<sub>max</sub> 3400.53, 2922.50, 1638.07, 1601.84, 820.57 cm<sup>-1</sup>. The <sup>1</sup>H-NMR (500 MHz in CDCl<sub>3</sub>) and <sup>1</sup>C-NMR (125 MHz in CDCl<sub>3</sub>) spectra were identical to those reported for astilbin.\textsuperscript{10}

**Compound 3** (Quercetin): C<sub>15</sub>H<sub>10</sub>O<sub>7</sub> (12.6 mg, 0.25 %w/w) was a yellow crystalline solid, with specific optical rotation [α]<sub>D</sub> = +28.07 (c 0.27, MeOH). This compound was compared with an authentic sample of quercetin (Merck) by TLC using 3 solvent systems and gave identical behaviour. The <sup>1</sup>H NMR spectrum agreed completely with published data for quercetin.\textsuperscript{11} Thus compound 3 was identified as quercetin.

**The anti-inflammatory effect**

The ethanolic extract of *Smilax corbularia* Kunth exhibited the most potent inhibitory activity on TNF-α and NO production, with an IC<sub>50</sub> value 61.97 and 83.90 µg/ml, whereas the water extract had no activity (IC<sub>50</sub> > 100 µg/ml) on any of the three pathways investigated (Table 1). Three flavonoid compounds, engeletin (compound 1), astilbin, (compound 2) and quercetin, (compound 3) were isolated from the ethanolic extract of this plant (Figure 1). Quercetin possessed the highest activity against NO production, with an IC<sub>50</sub> value of 11.2 µg/ml or 33.12 µM, whereas engeletin and astilbin had no effect on nitric oxide production (Tables 2-5). Engeletin, astilbin and quercetin all possessed potent inhibitory activity against PGE<sub>2</sub> release from RAW 264.7 cells with IC<sub>50</sub> values of 33.1, 43.5 and 65.8 µM, respectively. Engeletin (dihydro-kaempferol 3-β glucoside) exhibited the most potent inhibitory effect on LPS-induced PGE<sub>2</sub> release in RAW 264.7 cells. Quercetin possessed the most potent inhibitory activity against TNF-α release with an IC<sub>50</sub> value of 1.25 µg/ml or 4.14 µM, whereas, compounds 1 and 2 had no activity (Table 5). Indomethacin, the positive control, showed inhibitory effects on NO, TNF-α and PGE<sub>2</sub> release of 56.8, 143.7 and 2.8 µM respectively. Quercetin showed higher IC<sub>50</sub> values for inhibition of NO production and TNF-α release than indomethacin. However, quercetin showed lower inhibition of PGE<sub>2</sub> release than indomethacin.

![Figure 1. Structures of compound 1-3 isolated from *Smilax corbularia*](image)

**Table 1.** Percent inhibition at 100 µg/ml and IC<sub>50</sub> values\textsuperscript{a} of *Smilax corbularia* extract on NO, TNF-α and PGE<sub>2</sub> production.

<table>
<thead>
<tr>
<th>Production</th>
<th>Ethanol extract</th>
<th>Water extract</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>% inhibition</td>
<td>IC&lt;sub&gt;50&lt;/sub&gt;</td>
</tr>
<tr>
<td>NO</td>
<td>60.9 ± 4.2</td>
<td>83.9 ± 3.8</td>
</tr>
<tr>
<td>TNF-α</td>
<td>61.8 ± 0.4</td>
<td>61.9 ± 0.9</td>
</tr>
<tr>
<td>PGE&lt;sub&gt;2&lt;/sub&gt;</td>
<td>42.1 ± 2.8</td>
<td>&gt; 100</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Each value represents the mean ± S.E.M of three determinations.

The cytotoxic effect of extracts on RAW 264.7 cells was determined by MTT assay. The ethanolic and water extracts showed of cell survival percentages of 97.57 and 123.32%, respectively, at a concentration of 100 µg/ml.
The cytotoxic effect of extracts on RAW 264.7 cells was determined by MTT assay. Engeletin showed cell survival percentages of 62.74, 83.60, 88.27, 106.24 and 119.53% at concentrations of 100, 50, 20, 10, and 1 µg/ml, respectively.

Table 3. Percent inhibition at various concentrations and IC_{50} values of astilbin (compound 2) on NO, TNF-α and PGE_{2} production.

<table>
<thead>
<tr>
<th>Production</th>
<th>% inhibition at various concentrations</th>
<th>IC_{50} (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 µg/ml 10 µg/ml 20 µg/ml 50 µg/ml</td>
<td></td>
</tr>
<tr>
<td>NO</td>
<td>2.9±0.3 0.2±2.9 0.5±2.5 7.4±1.8</td>
<td>&gt;100</td>
</tr>
<tr>
<td>TNF-α</td>
<td>1.4±3.4 17.3±4.8 19.5±4.5 29.1±0.7</td>
<td>&gt;100</td>
</tr>
<tr>
<td>PGE_{2}</td>
<td>1.1±0.4 36.1±1.9 64.1±3.4 82.5±5.6</td>
<td>14±4.9</td>
</tr>
</tbody>
</table>

*Each value represents the mean ± S.E.M of three determinations.

From this study, quercetin (compound 3) is the active anti-inflammatory compound of *Smilax corbularia* that showed the strongest inhibitory effect on LPS-stimulation of three anti-inflammatory pathways.

**Discussion**

The water extract had no activity (IC_{50} > 100 µg/ml) on any of the three pathways investigated, this result does not agree with a previous report when an extract was tested for oestrogenic effects. However, there is no previous reports of astilbin and engeletin being anti-inflammatory compounds from *Smilax corbularia*. Astilbin and engeletin were isolated from the leaves of *Engelhardia roxburghiana* and studied for their effect in LPS-stimulated mouse J774A.1 macrophage cells. Engeletin and astilbin both exhibited remarkable inhibitory effects on interleukin (IL)-1β and IL-6 mRNA expression and were proposed to have potential anti-inflammatory properties. However, there is no report of these compounds having an inhibitory effect on LPS-stimulated NO, TNF-α and PGE_{2} release from RAW 264.7 cells. Measurement of the proteolytic activity of rabbit muscle 20S proteasomes showed that quercetin acted as a potent anti-inflammatory compound, respectively.

Quercetin (compound 3) isolated compound of *Smilax corbularia* is an active anti-inflammatory because it showed the strongest inhibitory effect on LPS-stimulation of three anti-inflammatory pathways. This result correlates with previous reports that demonstrated the anti-inflammatory effect of quercetin.

The constituents that were identified in the extract agree to some extent with those reported previously when an extract was tested for oestrogenic effects. However, there is no previous reports of astilbin and engeletin being anti-inflammatory compounds from *Smilax corbularia*. Astilbin and engeletin were isolated from the leaves of *Engelhardia roxburghiana* and studied for their effect in LPS-stimulated mouse J774A.1 macrophage cells. Engeletin and astilbin both exhibited remarkable inhibitory effects on interleukin (IL)-1β and IL-6 mRNA expression and were proposed to have potential anti-inflammatory properties. However, there is no report of these compounds having an inhibitory effect on LPS-stimulated NO, TNF-α and PGE_{2} release from RAW 264.7 cells. Measurement of the proteolytic activity of rabbit muscle 20S proteasomes showed that quercetin acted as a potent anti-inflammatory compound, respectively.

Table 4. Percent inhibition at various concentrations and IC_{50} values of quercetin (compound 3) on NO, TNF-α and PGE_{2} production.

<table>
<thead>
<tr>
<th>Production</th>
<th>% inhibition at various concentrations</th>
<th>IC_{50} (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.1 µg/ml 1 µg/ml 10 µg/ml 20 µg/ml</td>
<td></td>
</tr>
<tr>
<td>NO</td>
<td>3.1±3.9 5.8±1.9 45.7±1.4 67.4±0.8</td>
<td>11.2±0.6</td>
</tr>
<tr>
<td>TNF-α</td>
<td>15.6±1.9 43.8±3.8 69.8±3.7 86.6±2.6</td>
<td>1.25±0.2</td>
</tr>
<tr>
<td>PGE_{2}</td>
<td>0.43±0.3 1.2±0.2 14.6±0.9 50.6±0.4</td>
<td>19.9±0.01</td>
</tr>
</tbody>
</table>

*Each value represents the mean ± S.E.M of three determinations.

Table 5. Comparison between IC_{50} (µM) of compounds 1-3 and indomethacin as a positive control (N = 3).

<table>
<thead>
<tr>
<th>Production</th>
<th>IC_{50} (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 2 3</td>
</tr>
<tr>
<td>NO</td>
<td>&gt;100 &gt;100 37.1 56.8</td>
</tr>
<tr>
<td>TNF-α</td>
<td>&gt;100 &gt;100 4.14 143.7</td>
</tr>
<tr>
<td>PGE_{2}</td>
<td>33.1 43.5 65.8 2.8</td>
</tr>
</tbody>
</table>

*Each value represents the mean ± S.E.M of three determinations.

The cytotoxic effect of the extract on RAW 264.7 cells was determined by MTT assay. Astilbin showed survival percentages of 159.7, 148.1, 151.9 and 161.9% at concentrations of 50, 20, 10, and 1 µg/ml, respectively.
because it decreased proteasome-mediated proteolytic degradation of P-IκB protein, resulting in decreased translocation of activated NF-κB to the nucleus, and reduced transcription of TNF-α and iNOS. This finding supports our discovery that quercetin from *Smilax corbularia* is a potent anti-inflammatory molecule because of its inhibitory activity against three pro-inflammatory pathways (TNF-α, PGE2 and NO). These three compounds, quercetin, astilbin (dihydroquercetin-3-β-glucoside) and engeletin (deoxydihydroquercetin-3-β-rhamnoside), are all flavonoids. Neither astilbin nor engeletin showed any inhibitory effect on NO and TNF-α release, whereas quercetin showed the highest inhibitory effect on NO and TNF-α release (Table 4). However, quercetin and dihydroquercetin-3-β-glucoside (astilbin) showed a similar inhibitory effect on PGE2 release, leading to the conclusion that glycosidal dihydroflavonol analogues of quercetin cannot inhibit NO and TNF-α release but strongly inhibit PGE2 release. Quercetin, which has no rhamnose, showed the highest inhibitory effect on tumor necrosis factor-alpha (TNF-α) release with IC50 = 1.25 µg/ml (4.14 µM), which compares with a previous report of a reduction of TNF-α level.11 Engeletin (compound 1) is a dihydrokaempferol glycoside, and showed an inhibitory effect on PGE2 but no effect on NO and TNF-α release, thus acting similarly to astilbin. Thus, flavonols that are modified as glycosides and as a 2-3 dihydro-form have no inhibitory effect on NO and TNF-α release, but do have an inhibitory effect on PGE2 release. A comparison of the OH group of ring C of the flavonol structure found that the OH group of engeletin and astilbin showed different IC50 values. Engeletin has only one hydroxyl group on ring C compared with astilbin which has two, and showed a higher inhibitory effect on PGE2 release.

A previous study reported that a dose of 1600 mg/kg of the ethanolic extract of *S. corbularia* rhizomes administered orally significantly suppressed the paw oedema induced by carrageenan in rats; this 1600 mg dose gives astilbin, engeletin and quercetin contents of 15.68, 8.48 and 4 mg when calculated from the percentage yield of the compounds (0.98, 0.53 and 0.25%). This dose of the extract effectively gave an astilbin dose of more than 15 mg/kg. This study matched the report of Li17 where only 5 mg/kg of astilbin was orally administered to rats, and this dose effectively prevented the development of arteriosclerosis in allotransplants by inhibiting the proliferation of smooth muscles in the tunica intima and tunica media, and by reducing infiltration of inflammatory cells. However, astilbin is the main anti-inflammatory compound of *S. corbularia* for inhibition of PGE2 release; it was calculated as 1.078 g/kg of plant or 9.8 g/kg of plant extract. Thus, 510.2 mg of *S. corbularia* extract gave 5 mg of astilbin or a dose that was effective for preventing inflammation.17 In this study, the IC50 of the three active compounds showed inhibitory effects on PGE2 release at about 20 µg/ml, but 5 mg of astilbin was shown to be effective in rat, so the dose in *vitro* was 250 times more effective than in *vivo*. Thus, we conclude that 15 mg of astilbin or 1600 mg/kg of extract in rat are inflammatory doses, whereas 5 mg of astilbin or 510 mg of *S. corbularia* extract are doses for inflammation prevention.

We conclude that the ethanolic extract of *Smilax corbularia* Kunth shows in *vitro* evidence for a possible anti-inflammatory effect in *vivo*, with the bulk of the activity probably being due to quercetin (compound 3). This compound possesses a tripartite effect consisting of inhibition of NO production and both PGE2 and TNF-α release. Engeletin (compound 1) and astilbin (compound 2) exhibited a potent effect only on PGE2 release. These results support the traditional use of *Smilax corbularia* rhizome extract by Thai folk doctors for inflammatory conditions such as arthritis, cancer and postpartum pain reduction.

**Acknowledgements**

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**References**


