# 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 $IC_{50}$ 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 dermopathy, 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 lipopolysacharide (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

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Submitted date: 30/5/2012
Accepted date: 18/6/2012

been carried out on the mechanism of action for extracts and isolated compounds, in particular any inhibitory effect on lipopolysaccharide (LPS)-stimulated $PGE_2$ and TNF-α release and NO production by RAW 264.7 cells. Thus, this study aimed to evaluate the activity, relevant to any anti-inflammatory effect, of *Smilax corbularia* Kunth extracts, by determination of inhibitory effects on LPS-stimulated prostaglandin E2 ($PGE_2$) release or COX-II inhibition, TNF-α 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 *Smilax corbularia* Kunth (Smilacaceae) (Hua-Khao-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, Songkhla, Thailand; the voucher number is SKPA 179190315.

### *Preparation of plant extracts*

The rhizomes of *Smilax corbularia* were dried at 50°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 µm) 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 *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 E2 EIA Kit Monoclonal were purchased from Cayman Chemical Company and used according to the manufacturer's instructions. Mouse TNF-α 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 glutamine, penicillin (100 µg/ml), streptomycin (100 µg/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.[4,6] 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 µg/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 µl, 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 $IC_{50}$ values were determined graphically (n = 4):

Inhibition (%) = (A-B)×100/(A – C)

A − C: $NO_2$ − concentration (μM)

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

*Effects on LPS-stimulated $PGE_2$ release from RAW 264.7 cells*

The effect of extracts on LPS-stimulated $PGE_2$ production by the murine macrophage-like RAW 264.7 cell line was evaluated using a method modified from that previously reported.[4,7,8] Briefly, the RAW 264.7 cells were seeded in 24-well plates (2.5 × $10^5$ 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 $PGE_2$ was determined with a $PGE_2$ Enzyme Immuno-Assay Kit. The production of $PGE_2$ was measured relative to that of the control treatment.

*Effects on LPS-induced TNF-α release from RAW 264.7 cells*

The effect of extracts on the release of TNF-α from RAW 264.7 cell lines was evaluated using Quantikine mouse TNF-α ELISA test kit.[7] Briefly, the cells were seeded in 96-well plates with 1 × $10^5$ cells/well, and allowed to adhere for 1 h. After that, the medium was replaced with fresh medium containing 100 μg/ml of LPS, together with test samples at various concentrations, and incubated for 24 h. The supernatant (50 μl) was then transferred into a 96-well ELISA plate and TNF-α concentrations were determined. The inhibition of TNF-α production was calculated by the following equation and then $IC_{50}$ values were graphically determined:

Inhibition (%) = (A-B)×100/(A – C)

A − C: TNF-α − concentration (μM)

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

***Statistical analysis***

The results are expressed as the mean ± SEM of f three determinations. The $IC_{50}$ 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 mg, 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 [$^1H$ and $^{13}C$ on a Varian Unity Inova 500 spectrometer (500 MHz for $^1H$; 125 MHz for $^{13}C$)], 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): $C_{21}H_{22}O_{10}$ (26.30 mg, 0.53% w/w) was a yellow brown powder; it showed HREIMS *m/z* $[M]^+$ 434.1204 (Calc. for $C_{21}H_{22}O_{10}$ 434.1204), specific optical rotation [α]D = +11.50 (c 0.20, MeOH), UV (MeOH) λmax (logε) 329.80 (4.76), 292.50 (5.30), 217.92 (5.46) nm, IR (KBr disc) λmax 3369.91, 2922.50, 1638.73, 1587.09, 826.56 $cm^{-1}$. The $^1H$-NMR (500 MHz in $CDCl_3$) and $^{13}C$-NMR (125 MHz in $CDCl_3$) spectra were identical to those reported for engeletin.[9]

**Compound 2** (Astilbin): $C_{21}H_{22}O_{11}$ (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 $C_{21}H_{22}O_{11}$ 450.1167), specific optical rotation [α]D = -2.92 (c 0.25, MeOH), UV (MeOH) λmax (logε) 330.44 (4.41), 290.73 (4.99), 226.60 (5.02) and 214.94 (5.11) nm. IR (KBr disc)

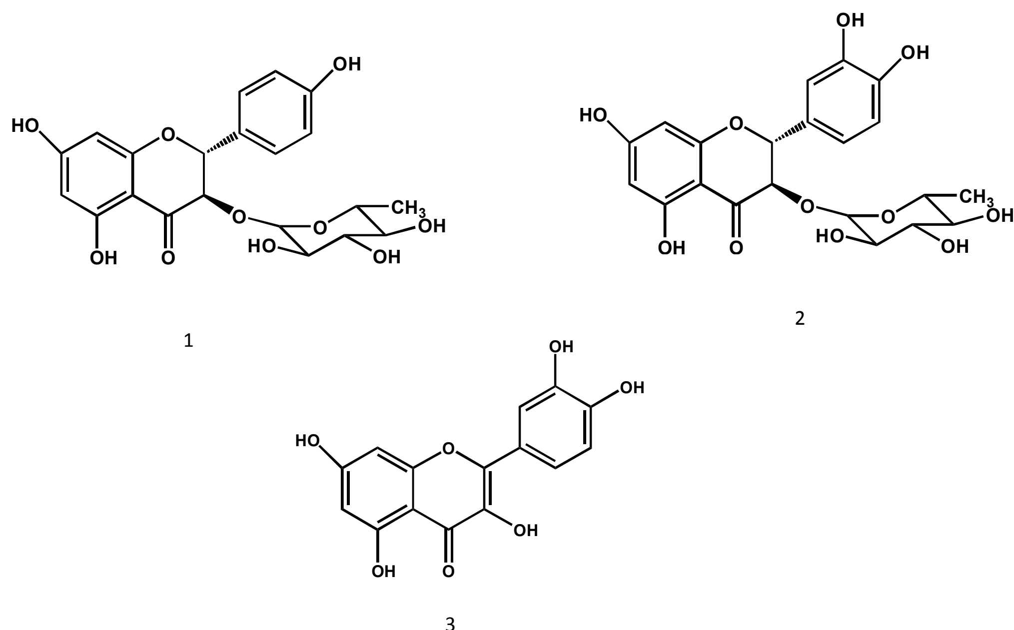


**Figure 1**. Structures of compound 1-3 isolated from *Smilax corbularia*

λmax 3400.53, 2922.50, 1638.07, 1601.84, 820.57 $cm^{-1}$. The $^{1}$H-NMR (500 MHz in $CDCl_3$) and $^{13}$C-NMR (125 MHz in $CDCl_3$) spectra were identical to those reported for astilbin.[10]

**Compound 3** (Quercetin): $C_{15}H_{10}O_7$ (12.6 mg, 0.25 %w/w) was a yellow crystalline solid, with specific optical rotation $[\alpha]_D$ = +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 $^{1}$H NMR spectrum agreed completely with published data for quercetin.[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 $IC_{50}$ value 61.97 and 83.90 µg/ml, whereas the water extract had no activity ($IC_{50}$ > 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 $IC_{50}$ 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 $PGE_2$ release from RAW 264.7 cells with $IC_{50}$ 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 $PGE_2$ release in RAW 264.7 cells. Quercetin possessed the most potent inhibitory activity against TNF-α release with an $IC_{50}$ 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 $PGE_2$ release of 56.8, 143.7 and 2.8 µM respectively. Quercetin showed higher $IC_{50}$ values for inhibition of NO production and TNF-α release than indomethacin. However, quercetin showed lower inhibition of $PGE_2$ release than indomethacin.

**Table 1**. Percent inhibition at 100 µg/ml and $IC_{50}$ values[a] of *Smilax corbularia* extract on NO, TNF-α and $PGE_2$ production.

| Production | EtOH extract | | Water extract | |
|---|---|---|---|---|
| | % inhibition at 100 µg/ml | $IC_{50}$ (µg/ml) | % inhibition at 100 µg/ml | $IC_{50}$ (µg/ml) |
| NO | 60.9 ± 4.2 | 83.9 ± 3.8 | 34.2 ± 4.9 | > 100 |
| TNF-α | 61.8 ± 0.4 | 61.9 ± 0.9 | 11.5 ± 10.2 | > 100 |
| $PGE_2$ | 42.1 ± 2.8 | > 100 | -1.6 ± 0.4 | > 100 |

[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.

**Table 2**. Percent inhibition at various concentrations and $IC_{50}$ values[a] of engeletin (compound 1) on NO, TNF-α and $PGE_2$ production.

| Production | % inhibition at various concentrations | | | | $IC_{50}$ |
|---|---|---|---|---|---|
| | 1 μg/ml | 10 μg/ml | 20 μg/ml | 50 μg/ml | (μg/ml) |
| NO | 2.9±0.3 | 0.2±2.9 | 0.5±2.5 | 7.4±1.8 | >100 |
| TNF-α | 1.4±3.4 | 17.3±4.8 | 19.5±4.5 | 29.1±0.7 | >100 |
| $PGE_2$ | 1.1±0.4 | 36.1±1.9 | 64.1±3.4 | 82.5±5.6 | 14.4±0.9 |

[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. 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 4.** Percent inhibition at various concentrations and $IC_{50}$ values[a] of quercetin (compound 3) on NO, TNF-α and $PGE_2$ production.

| Production | % inhibition at various concentrations | | | | $IC_{50}$ |
|---|---|---|---|---|---|
| | 0.1 μg/ml | 1 μg/ml | 10 μg/ml | 20 μg/ml | (μg/ml) |
| NO | 3.1±3.9 | 5.8±1.9 | 45.7±1.4 | 67.4±0.8 | 11.2±0.6 |
| TNF-α | 15.6±1.9 | 43.8±3.8 | 69.8±3.7 | 86.6±2.6 | 1.25±0.2 |
| $PGE_2$ | 0.43±0.3 | 1.2±0.2 | 14.6±0.9 | 50.6±0.4 | 19.9±0.01 |

[a]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. Quercetin showed cell survival percentages of 80.3, 116.0, 136.7 and 131.3% at concentrations of 20, 10, 1, and 0.1 μg/ml, respectively.

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 where the water extract showed an inhibitory effect on NO production ($IC_{50}$ = 61.0 μg/ml).[4] However, the material used in that study was collected in January, but in this work reported here, plants were collected in June, the rainy season in Thailand. Thai Traditional Medicine states that the rhizome should be collected only in the winter season, because then the rhizomes of plants stop growing and maintain a high level of active ingredients in the rhizome.[12]

**Table 3.** Percent inhibition at various concentrations and $IC_{50}$ values[a] of astilbin (compound 2) on NO, TNF-α and $PGE_2$ production.

| Production | % inhibition at various concentrations | | | | $IC_{50}$ |
|---|---|---|---|---|---|
| | 1 μg/ml | 10 μg/ml | 20 μg/ml | 50 μg/ml | (μg/ml) |
| NO | 3.4 ± 3.6 | 4.4 ± 1.6 | 5.2 ± 1.5 | 11.5±2.9 | > 100 |
| TNF-α | 17.9±3.9 | 14.1±3.8 | 18.3±1.4 | 27.2±1.1 | > 100 |
| $PGE_2$ | 18.7±0.8 | 20.2±0.5 | 51.3±0.3 | 89.9±0.4 | 19.6±0.01 |

[a]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.

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.[13-15]

The constituents that were identified in the extract agree to some extent with those reported previously when an extract was tested for oestrogenic effects.[5] 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.[16] 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.[16] 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,

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

| Production | $IC_{50}$ (μM) | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | Indomethacin |
| NO | > 100 | > 100 | 37.1 | 56.8 |
| TNF-α | > 100 | > 100 | 4.14 | 143.7 |
| $PGE_2$ | 33.1 | 43.5 | 65.8 | 2.8 |

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.[9] 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, but 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 $PGE_2$ release, leading to the conclusion that glycosidal dihydroflavonol analogues of quercetin cannot inhibit NO and TNF-α release but strongly inhibit $PGE_2$ release. Quercetin, which has no rhamnose, showed the highest inhibitory effect on tumor necrosis factor-alpha (TNF-α) release with $IC_{50}$ = 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 $PGE_2$ 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 $PGE_2$ 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 $IC_{50}$ values. Engeletin has only one hydroxyl group on ring C compared with astilbin which has two, and showed a higher inhibitory effect on $PGE_2$ 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[3]; 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 Li[17] 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 $PGE_2$ 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 $IC_{50}$ of the three active compounds showed inhibitory effects on $PGE_2$ release at about 20 μg/ml, but 5 mg of astilbin was shown to be effective in rat, so the dose *in vivo* was 250 times more effective than *in vitro*. 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 tri-partite effect consisting of inhibition of NO production and both $PGE_2$ and TNF-α release. Engeletin (compound 1) and astilbin (compound 2) exhibited a potent effect only on $PGE_2$ 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

A part of this work was financially supported by the National Research Council of Thailand and the National Research University Project of Thailand Office of Higher Education Commission, Thammasat University. We also thank Professor Peter Houghton from King's College, London for revision of this manuscript, and the Faculty of Medicine, Thammasat University, Thailand, for providing laboratory facilities.

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