Anti-Allergic Activities of Smilax glabra Rhizome Extracts and Its Isolated Compounds

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Background: The rhizomes of Smilax glabra (SG) has long been used in Traditional Chinese and Thai herbal medicine to treat a variety of infectious diseases and immunological disorders.

Objective: To investigate the in vitro anti-allergic activities of crude extracts and pure isolated flavonoid compounds from SG by determination of inhibitory effect on antigen-induced release of β-hexosaminidase from RBL-2H3 cells.

Material and Method: The in vitro inhibitory effects of crude aqueous and organic extracts on β-hexosaminidase release in RBL-2H3 cells were evaluated as an in vitro indication of possible anti-allergic activity. Bioassay-guided fractionation of extracts was used to isolate flavonoid compounds from the ethanolic extracts.

Results: The 95% and 50% ethanolic extracts of SG showed remarkably high anti-allergic activity, with IC50 values of 5.74 ± 2.44 and 23.54 ± 4.75 μg/ml, much higher activity than that for Ketotifen (IC50 58.90 μM). The water extract had negligible activity (IC50 >100 μg/ml). The two isolated flavonols, Engeletin and Astilbin, showed weak anti-allergic activity, IC50 values 97.46 ± 2.04 and >100 μg/ml, respectively.

Conclusion: The 95% and 50% ethanolic extracts of SG showed strong anti-allergic activity, but two flavonol constituents did not show any significant anti-allergic activity. These findings suggest that a combination of effects of various phytochemicals in crude extracts used in traditional medicine, are responsible for the purported anti-allergic activity of SG herbal preparations. The plethora of constituents in crude extracts, as yet unidentified, are likely to be acting synergistically to account for the strong observed anti-allergic in vitro activity.

Keywords: Smilax glabra, Anti-allergic activity, RBL-2H3 cells, Astilbin, Engeletin

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Allergy is a hypersensitivity disorder of the immune system, directed against environmental substances (allergens) and non-infectious products of certain infectious organisms. Immediate hypersensitivity (type I allergy), is an immunoglobulin E (IgE)-mediated immune response, resulting in conditions such as food allergies, hay fever, asthma and drug-induced allergies(1). A 2011 report by the World Allergy Organization (WAO) states, “The prevalence of allergic disease worldwide is rising dramatically in both developed and developing countries(2). These diseases include asthma; rhinitis; anaphylaxis; drug, food, and insect allergy; eczema; and urticaria (hives) and angiodema”. The reasons for this remarkable increase in prevalence rates of allergic disorders are still a matter of controversy(3). The ‘hygiene hypothesis’, which attributes a reduced microbial burden during childhood as a consequence of modern lifestyle, appears as a reasonable explanation. Surveys carried out in Thailand have revealed that allergic incidences have increased 3-4 fold over the last 40 years. In 2012, more than 18 million people in the country were diagnosed as suffering from some form of allergy. More than 10 million were diagnosed as suffering from allergic rhinitis (23-50%). Incidence rates for urticaria and dermatitis allergic (15%), asthma (10-15%) and food allergic (5%) were also high(4). These
allergic diseases can in some cases cause severe symptoms, and in all cases, they cause major disruption to quality of life.

A large number of modern procedures are available for the accurate diagnosis, prevention and treatment of allergic disorders, including drug treatment. However, specialized units within hospitals on allergy treatment are not always accessible in most countries. Furthermore, allergic disorders are chronic conditions that can affect multiple organs and systems throughout the lifespan of patients. The cost of these modern drugs and treatment procedures are prohibitive for majority of the world’s population, who, therefore, rely on low-cost traditional herbal medicines. Indeed, the World Health Organization (WHO) summarizes in its 2011 report that in much of the developing world, 70–95% of the population relies on such traditional medicines for primary healthcare.

Herbal medicines from Smilax, a genus of about 300–350 species native to tropical and template parts of the world, has been used in traditional medicine by most cultures over hundreds of years. For example, the roots of Smilax officinalis (common name sarsaparilla) was used in Central and South American ancient cultures, probably for centuries, before its discovery and subsequent introduction into European medicine around 1,550–1,600. Interestingly ancient cultures in Africa and Asia were also using other Smilax species for very similar conditions to those used by ancient South American cultures. The common name “Sarsaparilla” is often used as a catchall term for many species of Smilax around the world that are very similar, both in appearance and regarding their traditional medicine usage. Parts of plants from this genus have been used in traditional medicine for treatment of abscesses, boils, cystitis; arthritis rheumatism; syphilis, cancer, mercury poisoning, and dysentery.

Smilax glabra (SG) is commonly used as a herbal medicine in traditional Chinese and Thai medicine. It is locally known in Thai as ‘Khao-Yen’, and in Chinese as tufuling. The rhizome of this plant has long been used as a common ingredient in many traditional medicines in numerous Asian countries for detoxification, clearing heat, relieving dampness and easing joint movement. There has been recent interest in the scientific study of SG, and a number of studies describe the isolation and biological activities of SG constituents. The main constituents of SG are phenylpropanoids, flavonoids, plant steroids, saponins, and phenolic compounds. Bioactivity assessment suggest that SG extracts possess anti-inflammatory, immunomodulatory, anti-rheumatic, anti-cardiac hypertrophy, anti-diabetic, anti-bacterial, antifungal, anti-viral and anti-hepatocarcinogenic effects.

There are very few reports on anti-allergic effects of SG. An early (1982) clinical report on the use of Fujibitol (a poly herbal product containing 13 different plants, including SG) suggested that this product was clinically effective in the treatment of patients operated on for chronic sinusitis. Subsequent in vivo studies in mice and rats also suggested that Fujibitol may be effective for treatment of certain allergic disorders. Another animal study, on water extracts of SG, showed a remarkable inhibition of the delayed-type hypersensitivity reaction induced by picryl chloride, without suppressing humoral immune response in adjuvant-non-injected footpad of adjuvant arthritic rats. These human and animal studies are indicative that SG may contain constituents with anti-allergic activities. To the best of our knowledge, however, there are no systematic in vitro studies that unequivocally demonstrate this as this date.

The main objective of this preliminary study was to investigate the in vitro anti-allergic activities of crude extracts of the rhizome of SG by determining the inhibitory effects on the IgE-induced release of β-hexosaminidase in RBL-2H3 cells. A secondary objective was to isolate the two major flavonoids, Engeletin and Astilbin and to study their anti-allergic activities, since various flavonoids from different plants have already been demonstrated to have anti-oxidant and anti-inflammatory properties, which can also have an indirect effect on pathophysiology of allergic diseases.

Material and Method

Plant materials

The rhizomes of SG (Smilacaceae) (Khao-Yen-Tai) were collected from Phetchabun Province, Thailand in March 2010. The plant material was authenticated and a voucher specimen (No. SKP179190701) has been deposited in the herbarium of Southern Center of Thai Medicinal Plants at the Faculty of Pharmaceutical Sciences, Prince of Songkla University, Songkla, Thailand.

Chemicals

Minimum essential medium eagle (MEM) was purchased from Gibco (OK, USA). Anti-dinitrophenylated bovine albumin (DNP-BSA), anti-
DNP IgE (Monoclonal Anti-DNP), dimethyl sulfoxide (DMSO), ketotifen fumarate, 4-Nitrophenyl N-acetyl-β-D-glucosaminide (PNAG) were purchased from Sigma (MO, USA). Fetal bovine serum (FBS), penicillin-streptomycin (P/S) and phosphate buffer saline (PBS) were purchased from Biochrom (MA, USA). Sodium bicarbonate was from Foday Hung Bhd (Kuala Lumpur, MY); Silica Gel 60 (0.063-0.200 mm), Silica Gel 60 (0.040-0.063 mm), citric acid monohydrate was from Merck (Darmstadt, Germany); ethanol 95% (commercial grade) was from Sasol (Johannesburg, South Africa); distilled water (Milli-Q, ≥18 Mega Ohm) was from Milford (MA, USA); 24-well and 96-well microplates were purchased from Costar Corning (VA, USA).

**Instruments**

Chromatographic column (5.5x90 cm, glass) was purchased from Bechhai (BKK, Thailand). CO2 humidified incubator was purchased from Shel lab (OR, USA). Laminar airflow cabinet was purchased from Boss tech (BKK, Thailand). Microplate reader was purchased from Bio Tek (VT, USA).

**Extraction**

The rhizomes of SG were washed, cut in small pieces (1x1 cm), dried at 50°C (24 hours), ground into a 40 mesh particle size powder. The powdered rhizome was macerated with 95% and 50% ethanol, and water as ethanolic extract and water extract, respectively. The dried plant material (100 g) was macerated with 95% ethanol (300 ml, SGE-95) and 50% ethanol (300 ml, SGE-50) for 72 hours at room temperature and then filtered (Whatman No. 1 paper). The solvent was evaporated using a rotary evaporator at 45°C. The water extract (SGW) was obtained by boiling dried plant material (200 g) in 300 ml of distilled water for 30 minutes, and filtered as above. The water was removed by lyophilization. The extracts were dried to constant weight in a vacuum desicator, and stored in air-tight glass containers at -20°C until required for experimentation. The yield (% w/w) for these is provided in Table 1.

**Isolation of bioactive flavonoid compounds from Smilax glabra extracts**

Bioassay-guided fractionation was used for isolating active flavonoid compounds. Crude ethanolic extract (50 g) was dissolved in methanol and mixed the extract with dry powder of silica gel 60 (0.063-0.200 mm) 100 g, and applied to the top of the vacuum liquid chromatographic column (VLC, 10.6x20 cm) packed with silica gel 60 (0.063-0.200 mm). The column was sequentially eluted with five solvent systems of increasing polarity; hexane (1,000 ml, F1); hexane: chloroform (1:1 v/v, 2,000 ml, F2); chloroform (2,000 ml, F3); chloroform: methanol (1:1 v/v, 2,000 ml, F4) and methanol (2,000 ml, F5). The solvents in each of these five eluates were evaporated to dryness using a rotary film evaporatory at 45°C. These extracts were dried to constant weight in a vacuum desiccator and stored at

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### Table 1. Percent inhibition at various concentrations and IC\textsubscript{50} values\textsuperscript{*} of Smilax glabra extracts on the release of β-hexosaminidase from RBL-2H3 cells

<table>
<thead>
<tr>
<th>Sample</th>
<th>Yield (% w/w)</th>
<th>Inhibition (%) at various concentrations (μg/ml)</th>
<th>IC\textsubscript{50} (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>SGE-95</td>
<td>8.65</td>
<td>42.87±2.62</td>
<td>58.07±4.31</td>
</tr>
<tr>
<td>SGE-50</td>
<td>11.64</td>
<td>24.84±9.78</td>
<td>37.12±4.02</td>
</tr>
<tr>
<td>SGW</td>
<td>15.92</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>SG-F1</td>
<td>0.03</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>SG-F2</td>
<td>0.19</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>SG-F3</td>
<td>0.69</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>SG-F4</td>
<td>82.88</td>
<td>32.58±6.16</td>
<td>51.05±3.45</td>
</tr>
<tr>
<td>SG-F5</td>
<td>14.56</td>
<td>15.86±6.90</td>
<td>29.50±3.28</td>
</tr>
<tr>
<td>Ketotifen</td>
<td>-</td>
<td>14.89±0.69</td>
<td>29.46±1.96</td>
</tr>
</tbody>
</table>

\textsuperscript{*} Each value represents the mean ± SEM of three determination. % w/w of SGE95, SGE-50 and SGW are as percentage of starting dried powered rhizome. % w/w of SG-F1 to SG-F5 are as percentage of dried SGE-95 crude extract.

NT = not test.
-20°C in air-tight glass containers until required. The % yields (w/w) of these semi-pure extracts F1-F5 are given in Table 1.

The anti-allergic activity of each fraction was tested as described below. Fraction SG-F4 which showed the highest anti-allergic activity (IC<sub>50</sub> = 9.12 μg/ml), and the highest yield (82.88% w/w of starting weight of crude extract), was selected for the isolation of pure compounds by standard column chromatography. Fraction SG-F4 (5 g) was chromatographed over 150 g of silica gel 60 (0.040-0.063 mm) using 9:3:0.5 chloroform: methanol: water and afforded 1,500 ml, 6:4 (500 ml), 4:6 (500 ml), 2:8 (300 ml) and 0:8 (70 g) combined fractions (A-E) were obtained from pooling fractions 1-3, 4-5, 7-20, 21-45, 46-100 and the yields of semi-pure extracts on evaporation of solvents were 0.04, 0.08, 3.63, 0.73, and 0.53 g, respectively. Fraction C (from combined fractions 7-20, 3.63 g) was re-chromatographed over 150 g of silica gel 60 (0.040-0.063 mm) using chloroform and methanol gradient and collection of 5 ml fractions as follows; chloroform: methanol 8:2 (1,500 ml), 6:4 (500 ml), 4:6 (500 ml), 2:8 (300 ml) and methanol (300 ml), respectively. Combined fractions 18-27, 28-35 afforded two dried extracts, with yields of 0.018 and 0.43 g, respectively. The first (0.018 g) was subjected to recrystallisation using methanol to afford compound 1. The second (0.43 g) was re-chromatographed over 70 g of silica gel 60 (0.040-0.063 mm) using 9:3:0.5 chloroform: methanol: water and afforded compound 1 (2.5 mg, 13.89%, w/w). Compound 1 (2.5 mg, 13.89%, w/w) was a yellow-brown amorphous powder, and compound 2 was identified by the same methods and concluded to be Astilbin.

**Anti-allergic activity**

RBL-2H3 rat basophilic leukemia cells [cell No. CRL-2256 from American Type Culture Collection (ATCC CRL-2256)] were cultured in MEM medium supplemented with 15% FBS, penicillin 100 units/ml and streptomycin 100 μg/ml. The cells were maintained at 37°C in a 5% CO<sub>2</sub> atmosphere with 95% humidity. The cells were harvested with 0.05% trypsin-EDTA and diluted to a suspension in fresh medium before plating for experiments as described below.

**Inhibitory effects on the release of β-hexosaminidase (β-HEX) from RBL-2H3 cells**

Inhibitory effects of extracts on the release of β-hexosaminidase from RBL-2H3 cells was evaluated by a modified method as previously reported<sup>29</sup>. Briefly, RBL-2H3 cells were seeded in 24-well plates at a concentration of 2x10<sup>4</sup> cells/well and allowed to adhere for 1-2 h at 37°C in 5% CO<sub>2</sub>. The cells were then sensitized with 0.45 μg/ml anti-DNP-IgE and incubated 24 h at 37°C in 5% CO<sub>2</sub>. The cells were then washed twice with 400 μl of Siraganian buffer [buffer A, 119 mM NaCl, 5 mM KCl, 5.6 mM glucose, 0.4 mM MgCl<sub>2</sub>, 1 mM CaCl<sub>2</sub>, 25 mM piperazine-N, N′-bis (2-ethanesulfonic acid, PIPES), 0.1% bovine serum albumin (BSA) and 40 mM NaOH, pH 7.2] and then incubated in 160 μl of Siraganian buffer for 10 min at 37°C. After that, 20 μl of sample solution was added to each well and incubated for 10 min, followed by addition of 20 μl of stimulant (DNP-BSA, final concentration 10 μg/ml) at 37°C for 20 min to stimulate the cells to degranulate. The supernatant was transferred into a 96-well plate and incubated with 50 μl of substrate (1 mM p-nitrophenyl-N-acetyl-β-D-glucosaminide) in 0.1 M citrate buffer (pH 4.5) at 37°C for 1 h. The reaction was stopped by adding 200 μl of stop solution (0.1 M Na<sub>2</sub>CO<sub>3</sub>/NaHCO<sub>3</sub>, pH 10.0). The absorbance was measured with a microplate reader at 405 nm. The inhibition (%) of the release of β-hexosaminidase by the samples was calculated by the following equation, and IC<sub>50</sub> values were graphically determined.

\[
\text{Inhibition} \% = 1 - \frac{\text{T-B-N}}{\text{C-N}} \times 100
\]

Control (C) was DNP-BSA (+) and test sample (−); test (T) was DNP-BSA (+) and test sample (+); blank (B) was DNP-BSA (−) and test sample (+); normal (N) was DNP-BSA (−) and test sample (−). Ketotifen (fumarate salt), an anti-allergic drug, was used as a reference standard.

**Statistical analysis**

The results were expressed as the mean ± SEM of three determinations at each concentration for each sample. The IC<sub>50</sub> values were calculated using the
Results

Isolated pure flavonoid compounds from the ethanolic extract of Smilax glabra

**Compound 1** (Fig. 1, Engeletin)

C_{21}H_{22}O_{10} (2.5 mg, 13.89% w/w of fractions 18-27) was a yellow-brown amorphous 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) λ_{max} (logε) 329.80 (4.76), 292.50 (5.30), 217.92 (5.46) nm, IR (KBr disc) λ_{max} 3,369.91, 2,922.50, 1,638.73, 1,587.09, 826.56 cm^{-1}(12). The 1H-NMR (500 MHz in CDCl₃) and 13C-NMR (125 MHz in CDCl₃) spectra were identical to those reported for Engeletin(28).

**Compound 2** (Fig. 1, Astilbin)

C_{21}H_{22}O_{11} (21.1 mg, 4.91% w/w of fractions 28-35) 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) λ_{max} (logε) 330.44 (4.41), 290.73 (4.99), 226.60 (5.02) and 214.94 (5.11) nm. IR (KBr disc) λ_{max} 3,400.53, 2,922.50, 1,638.07, 1,601.84, 820.57 cm^{-1}(12). The 1H-NMR (500 MHz in CDCl₃) and 13C-NMR (125 MHz in CDCl₃) spectra were identical to those reported for Astilbin(28).

Inhibitory effects on the release of β-hexosaminidase from RBL-2H3 cells

The inhibitory effects of *Smilax glabra* extracts and two of its flavonoid compounds on IgE-induced degranulation in sensitized RBL-2H3 cells were examined. β-HEX was used as a marker of the degranulation of RBL-2H3 cells. As shown in Table 1, SGE-95 extract exhibited the most potent inhibitory activity on the release of β-HEX, with an IC₅₀ value of 5.74 µg/ml, followed by SG-F4 (IC₅₀ = 9.12 µg/ml), SGE-50 (IC₅₀ = 23.54 µg/ml) and SG-F5 (IC₅₀ = 30.47 µg/ml).

In contrast, SGW had no measurable activity (IC₅₀ >100 µg/ml). The anti-allergic activities of SGE-95, SG-F4 and SGE-50 were significantly higher than that of ketotifen, a reference standard (IC₅₀ = 25.06 µg/ml, 58.90 µM), whereas that of SG-F5 was slightly lower than Ketotifen and the other samples.

The two flavonoid compounds, Engeletin (compound 1) and Astilbin (compound 2) (Fig. 1) were isolated from the VLC fraction SG-F4. Their anti-allergic activities are shown in Table 2. Engeletin possessed low anti-allergic activity with an IC₅₀ value of 97.46 µg/ml (224.36 µM), whereas Astilbin was inactive (IC₅₀ >100 µg/ml), compared with the reference standard, ketotifen (IC₅₀ = 25.06 µg/ml, 58.90 µM).

The results clearly show that the ethanolic extracts of *Smilax glabra* exhibit much higher anti-allergic activities than ketotifen. The 95% ethanolic extract activity was the highest anti-allergic activity in this study, about 5 fold higher than for ketotifen. In addition, fraction SG-F4 not only showed higher anti-allergic effect than ketotifen, but it also showed higher effect than the 50% ethanolic extract. However, anti-allergic activity of fraction SG-F5 was similar to that of ketotifen.

It is interesting to note that the activities of the pure Engeletin and Astilbin were very low or negligible, compared to SG-F4, the semi-pure fraction (IC₅₀ 9.12 ±1.72 µg/ml) from which these two compounds were isolated. Clearly, activity is lost when SG-F4 is subjected to further purification.

Discussion

Anti-allergic activity of *Smilax glabra* extracts and pure constituents were tested by measuring their inhibitory effects on the release of β-hexosaminidase in IgE-sensitised and DNP-BSA stimulated rat basophilic leukemia RBL-2H3 cells. Direct measurement of histamine is complicated so the method for testing anti-allergic activity is the inhibitory effect on the release of β-hexosaminidase. β-hexosaminidase (β-HEX) is the enzyme which is released along with histamine when allergy occurs(30).

The results revealed that the water extract of SG had no anti-allergic activity (IC₅₀ >100 µg/ml) in this study which is type I hypersensitivity testing. Previous in vivo study in rats has reported that water extracts of *Smilax glabra* selectively inhibited the effector phase of the delayed-type hypersensitivity reaction(31).

The 95% ethanolic extract possessed the highest inhibition against release of β-hexosaminidase, followed by SG-F4, SGE-50 and SG-F5 (IC₅₀ = 5.74, 9.12,
Table 2. Percent inhibition at various concentrations and IC\textsubscript{50} values\textsuperscript{a} of Engeletin and Astilbin on the release of β-hexosaminidase from RBL-2H3 cells

<table>
<thead>
<tr>
<th>Sample</th>
<th>Inhibition (%) at various concentrations (μg/ml)</th>
<th>IC\textsubscript{50} (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>Engeletin</td>
<td>4.60±1.72</td>
<td>11.05±2.03</td>
</tr>
<tr>
<td>Astilbin</td>
<td>7.67±2.26</td>
<td>13.12±1.94</td>
</tr>
<tr>
<td>Ketotifen</td>
<td>14.89±0.69</td>
<td>29.46±1.96</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Each value represents the mean ± SEM of three determination

Engeletin and Astilbin were apparently inactive against the enzyme activity of β-hexosaminidase, they possess antioxidant and anti-inflammatory properties. Their anti-inflammatory activities were studied in vitro using mouse J774A.1 macrophage cells stimulated with lipopolysaccharide (LPS). Engeletin and astilbin exhibited notable inhibitory effects on interleukin (IL)-1B and IL-6 mRNA expression\textsuperscript{(32)}. This reduction in cytokines expression may reduce the immune response and inflammatory activation. A recent study reported that both Engeletin and Astilbin exhibited inhibitory effect on prostaglandin E2 (PGE\textsubscript{2}) release from RAW 264.7 cells, with an IC\textsubscript{50} values of 14.4 and 19.6 μg/ml, respectively. In in vitro studies, PGE\textsubscript{2} play an important role in enhancing IL-4 and IL-5 production\textsuperscript{(33)}. These mediators, especially IL-4, releasing from the cells are involved in the late-phase reaction in type I allergy.

The finding indicates that the 95% ethanolic extract of SG has the highest anti-allergic activity, but the two main flavonoid components, Engeletin and Astilbin had no anti-allergic effect. Thus, the 95% ethanolic extract of Smilax glabra has the potential for development as a food supplement or functional food, for treating allergic disorders. However, further studies are necessary to isolate a range of bioactive compounds from the water and ethanolic extracts of SG.

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Potential conflicts of interest
None.

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คุณภาพสารสังเคราะห์ของสมิลักกลรา และสารปริมาตรจากหน้าขาวเย็นได้

อรุณทร อดุลทัศน์, ถนอมชัย ศรีนิช, ศรีนิช เรืองทุ่ม, ผลการพอใจ

ภูมิตรด้วย: หน้าขาวเย็นได้ชื่อ Smilax glabra (Smilacaceae) เป็นสมุนไพรที่ใช้ในหลายด้านในประเทศไทย และประเทศอื่น โดยเฉพาะใช้วาด
โรคติดเชื้อด้วยและโรคในระบบกระดูก

วัสดุและวิธีการ: ยกสกัดจากหน้าขาวเย็นได้ชื่อ Smilax glabra ด้วย 95, 50 เปอร์เซ็นต์เอทานอลและน้ำ แยกสารปริมาตรโดยใช้ bio assay
guided isolation และทดสอบสารสังเคราะห์จากเย็นได้ชื่อ β-hexosaminidase จากเซลล์ RBL-2H3

ผลการค้นหา: สารสกัดหัวใจเย็นได้ชื่อเกิดตัวออกของเย็น 95% เท่านั้น 50% และอุปกรณ์ในการพบค่า IC₀ = 5.74±2.44 และ 23.54±4.75
µg/ml ตามลำดับ ซึ่งมีที่สว่างมากกว่าของ ketotifen fumarate (IC₅₀ = 25.06±2.02 µg/ml) ในเซลล์กระดูกเย็นได้ไม่ถูก (IC₅₀ >100
µg/ml) สามารถจัดตั้งได้เป็นสาร flavonol 2 สาร คือ astilbin และ engeletin มีคุณสมบัติการพบขั้นต่ำ IC₅₀ = 97.46±2.04 และ >100
µg/ml ตามลำดับ

สรุป: สารสกัดหัวใจเย็นได้ดุลออกของเย็น 95% มีคุณสมบัติการพบขั้นต่ำสูงที่เป็น flavonol ในมีอยู่ในสารสกัด ผลการวิจัยขั้นต่ำ
แสดงว่าสารสกัดจากหน้าขาวเย็นได้ชื่อ Smilax glabra มีคุณสมบัติในการพบขั้นต่ำในสารสกัดที่พบไม่มีฝุ่นด้านเย็นได้ ซึ่งผลของการใช้สารสกัดเวนได้การรักษาโรคฝุ่นทดแทนของแพทย์แผนไทย
การนี้สารสกัดในสารสกัดเหมาะสมกับและทดสอบคำว่ามีที่สามารถพบขั้นต่ำหรือไม่ควรทำการศึกษาถึง