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

Arunporn Itharat PhD*,***, Kamonmas Srikwan BSc**,
Srisopa Ruangnoo PhD*, Pakakrong Thongdeeying MSc*

** Department of Applied Thai Traditional Medicine, Faculty of Medicine, Thammasat University, Pathumthani, Thailand*
*** Student of Master Degree in Medical Science Program, Nutraceuticals, Faculty of Medicine, Thammasat University, Pathumthani, Thailand*
**** Center of Excellence in Applied Thai Traditional Medicine Research (CEATMR), Thammasat University, Pathumthani, Thailand*

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 IC_{50} values of 5.74±2.44 and 23.54±4.75 μg/ml, much higher activity than that for Ketotifen (IC_{50} 58.90 μM). The water extract had negligible activity (IC_{50} >100 μg/ml). The two isolated flavonols, Engeletin and Astilbin, showed weak anti-allergic activity, IC_{50} 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*

J Med Assoc Thai 2015; 98 (Suppl. 3): S66-S74
Full text. e-Journal: *http://www.jmatonline.com*

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 controvercy[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

Correspondence to:
Itharat A, Department of Applied Thai Traditional Medicine, Faculty of Medicine, Thammasat University, Rangsit, Klongluang, Pathumthani 12120, Thailand.
Phone & Fax: +66-2-9269749
E-mail: iarunporn@yahoo.com

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[5].

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[6]. 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[7-11].

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[12,13]. 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[14-17]. 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[14,18-22].

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[23]. Subsequent in vivo studies in mice and rats also suggested that Fujibitol may be effective for treatment of certain allergic disorders[24,25]. 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[26]. 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[27], 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 Becthai (BKK, Thailand). CO_2 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 lyophillization. The extracts were dried to constant weight in a vacuum desiccator, 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

Table 1. Percent inhibition at various concentrations and IC_{50} values[a] of *Smilax glabra* extracts on the release of β-hexosaminidase from RBL-2H3 cells

Sample	Yield (% w/w)	Inhibition (%) at various concentrations (μg/ml)				IC_{50} (μg/ml)
		1	10	50	100	
SGE-95	8.65	42.87±2.62	58.07±4.31	78.14±1.16	76.67±1.78	5.74±2.44
SGE-50	11.64	24.84±9.78	37.12±4.02	60.95±4.72	65.66±5.33	23.54±4.75
SGW	15.92	NT	NT	NT	33.56±3.46	>100
SG-F1	0.03	NT	NT	NT	NT	NT
SG-F2	0.19	NT	NT	NT	NT	NT
SG-F3	0.69	NT	NT	NT	NT	NT
SG-F4	82.88	32.58±6.16	51.05±3.45	76.28±1.40	83.86±0.66	9.12±1.72
SG-F5	14.56	15.86±6.90	29.50±3.28	59.96±1.42	69.08±5.40	30.47±3.80
Ketotifen	-	14.89±0.69	29.46±1.96	72.77±0.90	89.11±1.43	25.06±2.02 (58.90 μM)

[a] 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_{50} = 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 1,000 ml of chloroform: methanol (1:1 v/v). Fractions (10 ml) were collected and examined by thin layer chromatography (TLC), with detection of compounds using anisaldehyde spraying reagent. Fractions showing the same TLC patterns were combined. Five separate 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 a 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 2. Compound 1 (2.5 mg, 13.89%, w/w) was a yellow-brown amorphous powder, and compound 2 (21.1 mg, 4.91%, w/w) was obtained as white crystals when crystallized from methanol. The structures of the isolated compounds (Fig. 1) were established by comparing the ^{1}H and ^{13}C NMR, and other spectral data with those previously reported. They were also compared with authentic compounds, kindly provided by Dr. Srisopa Reuangnoo[28]. Purity was ensured by chromatography using three TLC solvent systems. Compound 1 had identical ^{1}H and ^{13}C NMR spectral to that of Engeletin, 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_2 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 detailed 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[29]. Briefly, RBL-2H3 cells were seeded in 24-well plates at a concentration of $2x10^5$ cells/well and allowed to adhere for 1-2 h at 37°C in 5% CO_2. The cells were then sensitized with 0.45 µg/ml anti-DNP-IgE and incubated 24 h at 37°C in 5% CO_2. 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 $MgCl_2$, 1 mM $CaCl_2$, 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_2CO_3/$NaHCO_3$, 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_{50} 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_{50} values were calculated using the

Prism program version 4.03 (Graphpad, San Diego, California).

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) λmax (logε) 329.80 (4.76), 292.50 (5.30), 217.92 (5.46) nm, IR (KBrdisc) λmax 3,369.91, 2,922.50, 1,638.73, 1,587.09, 826.56 cm^{-1}[12]. 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 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) λmax (logε) 330.44 (4.41), 290.73 (4.99), 226.60 (5.02) and 214.94 (5.11) nm. IR (KBr disc) λmax 3,400.53, 2,922.50, 1,638.07, 1,601.84, 820.57 cm^{-1}[12]. The ^{1}H-NMR (500 MHz in $CDCl_3$) and ^{13}CNMR (125 MHz in $CDCl_3$) 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 IC_{50} value of 5.74 μg/ml, followed by SG-F4 (IC_{50} = 9.12 μg/ml), SGE-50 (IC_{50} = 23.54 μg/ml) and SG-F5 (IC_{50} = 30.47 μg/ml).

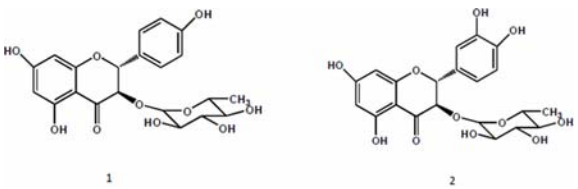

Fig. 1 Chemical structures of compound 1 and 2 isolated from *Smilax glabra.*

In contrast, SGW had no measurable activity (IC_{50} >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 (IC_{50} = 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 IC_{50} value of 97.46 μg/ml (224.36 μM), whereas Astilbin was inactive (IC_{50} >100 μg/ml), compared with the reference standard, ketotifen (IC_{50} = 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 (IC_{50} 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 (IC_{50} >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 (IC_{50} = 5.74, 9.12,

Table 2. Percent inhibition at various concentrations and IC_{50} values[a] of Engeletin and Astilbin on the release of β-hexosaminidase from RBL-2H3 cells

Sample	Inhibition (%) at various concentrations (μg/ml)				IC_{50} (μg/ml)
	1	10	50	100	
Engeletin	4.60±1.72	11.05±2.03	28.67±3.03	51.73±1.54	97.46±2.04 (224.36 μM)
Astilbin	7.67±2.26	13.12±1.94	32.74±7.00	48.37±1.37	>100
Ketotifen	14.89±0.69	29.46±1.96	72.77±0.90	89.11±1.43	25.06±2.02 (58.90 μM)

[a] Each value represents the mean ± SEM of three determination

23.54 and 30.47 μg/ml, respectively), compared with the reference standard, ketotifen (IC_{50} = 25.06 μg/ml, 58.90 μM). Interestingly 95% ethanolic extract showed the highest anti-allergic activity, but this activity decreased when this crude extract was subjected to semi-purification to obtain F1-F5 semi-pure extracts. These results suggest that constituents in the 95% ethanolic extract were acting synergistically. Engeletin and Astilbin (Fig. 1), the major flavonoid compounds isolated from SG rhizome extract were also tested for anti-allergic effect. The results demonstrated that both had weak or negligible anti-allergic activity. SG extracts, like or species in *Smilax*, probably contain a wide range of constituents, including at least five flavonoids. Several of these flavonoids, plus other phenolic compounds, are likely to be acting in synergy to account for these observations.

Although Engeletin and Astilbin were apparently inactive against the enzyme activity of β-hexosaminidase, they possess antioxidant and anti-inflammation 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)-1β and IL-6 mRNA expression[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_2) release from RAW 264.7 cells, with an IC_{50} values of 14.4 and 19.6 μg/ml, respectively. In in vitro studies, PGE_2 play an important role in enhancing IL-4 and IL-5 production[33]. These mediators, especially IL-4, releasing from the cells are involved in the late-phase reaction in type I allergy. The late-phase reaction occurs within 4-6 hours after the early-phase reaction in type I allergy. It implies that Engeletin and Astilbin may serve as potential anti-inflammatory agents in the late-phase reaction. In addition, oxidative stress plays a substantial role in the pathophysiology of allergic diseases. Exogenous of reactive oxygen species (ROS) promote the inflammatory process by inducing the release of pro-inflammatory mediator, such as cytokines and chemokines, and activating gene expression[34]. Engeletin and Astilbin showed high antioxidant activity by the DPPH radical scavenging assay with EC_{50} of 3.9 and 2.2 μg/ml, respectively[35]. Moreover Engeletin and Astilbin was also studied using the lipid per-oxidation of liposome assay[35]. The EC_{50} values of Engeletin and Astilbin were found to be 1.2 and 0.8 μg/ml, respectively[35].

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.

Acknowledgement

This study was in part supported by the National Research University Project of Thailand Office of Higher Education Commission (NRU). We also thank the Faculty of Medicine, Thammasat University, Thailand for providing laboratory facilities, and Professor L. A. Damani, visiting Professor at Thammasat University, for his critical comments and careful scientific editing of this manuscript.

Potential conflicts of interest

None.

References

1. Galli SJ, Tsai M, Piliponsky AM. The development of allergic inflammation. Nature 2008; 454: 445-54.
2. Pawankar R, Canonica GW, Holgate ST, Lockey RF. WAO white book on allergy 2011-2012: executive summary [Internet]. 2011 [updated 2012 Apr 23; cited 2013 Nov 27]. Available from: http://www.worldallergy.org/publications/wao_white_book.pdf
3. Romagnani S. The increased prevalence of allergy and the hygiene hypothesis: missing immune deviation, reduced immune suppression, or both? Immunology 2004; 112: 352-63.
4. Bunjean K, Sukkasem K, Noppacroh N, Yamkaew N, Janthayanont D, Theerapancharern W, et al. Prevalence of allergic rhinitis and types of sensitized allergen in adult at Wat Intaram community, Hua Raeu, Phra Nakhon Si Ayutthaya District, Phra Nakhon Si Ayutthaya Province, Thailand. J Med Assoc Thai 2012; 95 (Suppl 5): S63-8.
5. Robinson MM, Zhang X. The World medicines situation [Internet]. 2011. [cited 2013 Nov 27]. Available from: http://www.who.int/medicines/areas/policy/world_medicines_situation/WMS_ch6_wPricing_v6.pdf.
6. Taylor L. The healing power of rainforest herbs. New York: Squareone; 2005.
7. Duke JA, Ayensu ES. Medicinal plants of China. Michigan: Algonac; 1985.
8. Fogarty JE. A Barefoot Doctor's manual. Marblemount, WA: META; 1974.
9. Yeung HC. Handbook of Chinese herbal formulas. 2nd ed. Taos, NM: Redwing Book; 1985.
10. Bown D. Encyclopedia of herbs and their uses. London: DK Publishing; 1995.
11. Kariyone T. Atlas of medicinal plants. Osaka, Japan: Takeda Chemical Industries; 1971.
12. Song L, Wu Y, Hu L, Zhang G, Xu G, Xiao P, et al. Zhong Hua Ben Cao. Shanghai, China: Shanghai Scientific Technologic Publisher. China Herbal 1999; 5: 859-67.
13. PRC PCo. In Pharmacopoeia of the People's Republic of China. Beijing, China: Chinese Medicine Science and Technology; 2010.
14. Zhang QF, Zhang ZR, Cheung HY. Antioxidant activity of Rhizoma Smilacis Glabrae extracts and its key constituent-astilbin. Food Chem 2009; 115: 297-303.
15. Chen L, Yin Y, Yi H, Xu Q, Chen T. Simultaneous quantification of five major bioactive flavonoids in Rhizoma smilacis glabrae by high-performance liquid chromatography. J Pharm Biomed Anal 2007; 43: 1715-20.
16. Yiqing L, Yanghua Y, Haifeng T, Kai X. Studies on the chemical constituents of Glabrous greenbrier (Smilax glabra). Chinese Traditional and Herbal Drugs 1996; 27: 712-4.
17. Xu S, Shang MY, Liu GX, Xu F, Wang X, Shou CC, et al. Chemical constituents from the rhizomes of Smilax glabra and their antimicrobial activity. Molecules 2013; 18: 5265-87.
18. Shou Q, Pan S, Tu J, Jiang J, Ling Y, Cai Y, et al. Modulation effect of Smilax glabra flavonoids on ryanodine receptor mediated intracellular Ca2+ release in cardiomyoblast cells. J Ethnopharmacol 2013; 150: 389-92.
19. Solomon Raju BG, Ganga Rao B, Manju Latha YB. Antidiabetic activity of Smilax china roots in alloxan-induced diabetic rats. Pharm Tech Res 2012; 4: 369-74.
20. Galhena PB, Samarakoon SR, Thabrew MI, Weerasinghe GA, Thammitiyagodage MG, Ratnasooriya WD, et al. Anti-inflammatory activity is a possible mechanism by which the polyherbal formulation comprised of Nigella sativa (Seeds), Hemidesmus indicus (Root), and Smilax glabra (Rhizome) mediates its antihepatocarcinogenic effects. Evid Based Complement Alternat Med 2012; 2012: 108626.
21. Ooi LS, Wong EY, Chiu LC, Sun SS, Ooi VE. Antiviral and anti-proliferative glycoproteins from the rhizome of Smilax glabra Roxb (Liliaceae). Am J Chin Med 2008; 36: 185-95.
22. Mendsaikhan Z, Gan-Urnuh B, Olzvoi T, Doljinsuren D, Purevjav M, Ariunaa Z, et al. Preliminary results of smilax glabra remedy usage for reumatoid patients. Mongolian Med 2009; 3: 70-3.
23. Toriyama Y. A Clinical Study of Fuji-Bitol in Patients operated on for Paranasal Sinusitis. Practica Oto-Rhino-Laryngologica 1982; 75: 1867-73.
24. Kakimoto M, Takasugi N, Fuwa T, Saito H, Sugimoto Y, Kamei C. Effects of fujibitol, a remedy for nasal symptoms of immediate and delayed type allergic reactions. Methods Find Exp Clin Pharmacol 1999; 21: 353-6.
25. Kakimoto M, Sugimoto Y, Harada M, Kobayashi Y, Okuma C, Taga C, et al. Effects of fujibitol, a remedy

for nasal symptoms, on experimental allergic rhinitis in rats. Biol Pharm Bull 2000; 23: 1055-8.
26. Jiang J, Xu Q. Immunomodulatory activity of the aqueous extract from rhizome of Smilax glabra in the later phase of adjuvant-induced arthritis in rats. J Ethnopharmacol 2003; 85: 53-9.
27. Kumar S, Pandey AK. Chemistry and biological activities of flavonoids: an overview. Scientific World Journal 2013; 2013: 162750.
28. Ruangnoo S, Jaiaree N, Makchuchit S, Panthong S, Thongdeeying P, Itharat A. An in vitro inhibitory effect on RAW 264.7 cells by anti-inflammatory compounds from Smilax corbularia Kunth. Asian Pac J Allergy Immunol 2012; 30: 268-74.
29. Lim H, Lee SY, Lee KR, Kim YS, Kim HP. The Rhizomes of Acorus gramineus and the Constituents Inhibit Allergic Response In vitro and In vivo. Biomol Ther (Seoul) 2012; 20: 477-81.
30. Yodsaoue O, Cheenpracha S, Karalai C, Ponglimanont C, Tewtrakul S. Anti-allergic activity of principles from the roots and heartwood of Caesalpinia sappan on antigen-induced beta-hexosaminidase release. Phytother Res 2009; 23: 1028-31.
31. Jiang J, Wu F, Lu J, Lu Z, Xu Q. Anti-inflammatory activity of the aqueous extract from Rhizoma smilacis glabrae. Pharmacol Res 1997; 36: 309-14.
32. Huang H, Cheng Z, Shi H, Xin W, Wang TT, Yu LL. Isolation and characterization of two flavonoids, engeletin and astilbin, from the leaves of Engelhardia roxburghiana and their potential anti-inflammatory properties. J Agric Food Chem 2011; 59: 4562-9.
33. Fedyk ER, Phipps RP. Prostaglandin E2 receptors of the EP2 and EP4 subtypes regulate activation and differentiation of mouse B lymphocytes to IgE-secreting cells. Proc Natl Acad Sci U S A 1996; 93: 10978-83.
34. Lee IT, Yang CM. Role of NADPH oxidase/ROS in pro-inflammatory mediators-induced airway and pulmonary diseases. Biochem Pharmacol 2012; 84: 581-90.
35. Itharat A, Kejik R, Tewtrakul S, Watanaperomskul C. Antioxidant and anti-HIV-1 integrase compounds from *Smilax corbularia* Kunth. Planta Med 2007; 73: 996-7.

ฤทธิ์ต้านการแพ้ของสารสกัดและสารบริสุทธิ์จากหัวข้าวเย็นใต้

อรุณพร อิฐรัตน์, กมลมาศ ศรีขวัญ, ศรีโสภา เรืองหนู, ผกากรอง ทองดียิ่ง

ภูมิหลัง: *หัวข้าวเย็นใต้ชนิด Smilax glabra (Smilacaceae) เป็นสมุนไพรที่ใช้ในหลายตำรับในประเทศจีน และประเทศไทย โดยเฉพาะใช้รักษาโรคติดเชื้อต่างๆ และโรคในระบบภูมิคุ้มกัน*

วัตถุประสงค์: *เพื่อศึกษาฤทธิ์ต้านการแพ้ ของสารสกัดและสารบริสุทธิ์จากหัวข้าวเย็นใต้ชนิด Smilax glabra โดยวิธียับยั้งการหลั่ง β-hexosaminidase จากเซลล์ RBL-2H3*

วัสดุและวิธีการ: *สกัดสารจากหัวข้าวเย็นใต้ชนิด Smilax glabra ด้วย 95, 50 เปอร์เซ็นต์เอทธานอลและน้ำ แยกสารบริสุทธิ์โดยวิธี bio assay guided isolation และทดสอบฤทธิ์ต้านการแพ้โดยการยับยั้งการหลั่ง β-hexosaminidase จากเซลล์ RBL-2H3*

ผลการศึกษา: *สารสกัดหัวข้าวเย็นใต้ที่สกัดด้วยด้วยเอทธานอล 95% เอทธานอล 50% แสดงฤทธิ์ต้านการแพ้มีค่า IC_{50} = 5.74±2.44 และ 23.54±4.75 μg/ml ตามลำดับ ซึ่งมีฤทธิ์ดีกว่าสารมาตรฐาน ketotifen fumarate (IC_{50} = 25.06±2.02 μg/ml) ในขณะที่สารสกัดชั้นน้ำไม่มีฤทธิ์ (IC_{50} >100 μg/ml) สารบริสุทธิ์ที่แยกได้เป็นสาร flavonol 2 สาร คือ astilbin และ engeletin มีฤทธิ์ต้านการแพ้น้อยมีค่า IC_{50} = 97.46±2.04 และ >100 μg/ml ตามลำดับ*

สรุป: *สารสกัดหัวข้าวเย็นใต้ด้วยเอทธานอล 95% มีฤทธิ์ต้านการแพ้ดีแต่สารสำคัญที่เป็น flavonol ไม่มีฤทธิ์ต้านการแพ้ ผลการวิจัยนี้แสดงว่าผลของการรวมตัวของสารสำคัญในสารสกัดหยาบทำให้มีฤทธิ์ต้านภูมิแพ้ ซึ่งสอดคล้องกับการใช้หัวข้าวเย็นใต้ในการรักษาโรคภูมิแพ้ของแพทย์แผนไทย การนำสารสำคัญในสารสกัดหยาบมารวมกันและทดสอบว่ามีฤทธิ์ต้านการแพ้เพิ่มขึ้นหรือไม่ควรนำมาศึกษาต่อไป*