Anti-Allergic, Anti-Inflammatory and Antioxidant Activities of the Different Extracts of Thai Traditional Remedy Called Prabchompoothaweep for Allergic Rhinitis Treatment

Areeratna Jai-aue BATM*, Sunita Makchuchit MSc*,
Thana Juckmeta MSc*, Arunporn Itharat PhD**.***

** Graduate School, Faculty of Medicine, Thammasat University, Pathumthani, Thailand*
*** Department of Applied Thai Traditional Medicine, Faculty of Medicine, Thammasat University, Pathumthani, Thailand*
**** Center of Excellence on Applied Thai Traditional Medicine Research (CEATMR), Faculty of Medicine, Thammasat University, Pathumthani, Thailand*

Background: *Prabchompoothaweep remedy (PT) has long been used in Thai traditional medicine to treat allergic rhinitis and asthma. It is composed of 23 plants. It is on National herbal drug list of Thailand, but there is no report for anti-allergic, anti-inflammatory and antioxidant activities.*
Objective: *To investigate anti-allergic, anti-inflammatory and antioxidant activities of the crude extract from PT by different extraction method.*
Material and Method: *The method of extract used was maceration in 95% ethanol and 50% ethanol; the residue of these extracts were continued extracted by boiling water, they obtained PTE95, PTE50, PTR95 and PTR50, respectively. The other method of extraction was boiling and drying by lyophilizer that obtained PTW. Five crude extracts were determined anti-allergic activity by the inhibition of β-hexosaminidase release from RBL-2H3 cell lines, anti-inflammatory activity were determined by the inhibition of nitric oxide (NO) production from RAW 264.7 cell lines induced by lipopolysaccharide (LPS) and antioxidant activity were tested by DPPH radical scavenging assay.*
Results: *PTE95 showed the most potent of anti-allergic activity, anti-inflammatory activity and antioxidant activity (IC_{50} = 12.97, 22.51 and EC_{50} = 14.62 μg/ml, respectively).*
Conclusion: *These results suggest that the method of extraction PT that showed the best anti-allergy, anti-inflamation and antioxidant activity was maceration in 95% ethanol.*

Keywords: *Anti-allergic activity, β-hexosaminidase, Anti-inflammatory activity, Nitric oxide, Antioxidant activity, Prabchompoothaweep remedy, Extract*

J Med Assoc Thai 2014; 97 (Suppl. 8): S140-S148
Full text. e-Journal: *http://www.jmatonline.com*

Allergy is a hypersensitivity of immunological reaction to allergens such as pollen, food, animal hair and dust mites which cause tissue inflammation and organ dysfunction[1]. The WAO (World Allergy Organization) reported the prevalence of allergic diseases in rising dramatically in both developed and underdeveloped countries[2]. The prevalence rate continues increasing worldwide and effects to 10-25% of the world population[3]. In Thailand, the prevalence of rhinitis in children was found about 14-44%, while in adults was found about 20%[4].

Allergic rhinitis associate with asthma. Patients with allergic rhinitis are high risk to developing bronchial and airway inflammation[5]. Allergic rhinitis (AR) results from an IgE-mediated inflammation of the nasal mucosa while asthma is a chronic inflammatory disorder of the lower airways. Mast cells are important role in hypersensitivity reaction. An allergen stimulates IgE antibody to bide with high affinity receptors (FcεRI) on mast cell and basophiles, lead to degranulation process that releases pro-inflammatory cytokines and mediators. This is causing of allergy and inflammatory

Correspondence to:
Itharat A, Department of Applied Thai Traditional Medicine, Faculty of Medicine, Thammasat University, Pathumthani 12120, Thailand.
Phone: 0-2926-9749
E-mail: iarunporn@yahoo.com

response[6]. During the degranulation process, β-hexosaminidase released by granules on mast cell and basophils that related with histamine and reported to be marker for determining the degree of degranulation[7,8]. Nowadays, people are interested to use medicinal plants and herbal products for quality of life considerations instead of chemical drug. Therefore, Prabchompoothaweep remedy in Thai traditional medicine has long been used to treat allergic rhinitis and asthma[9] and should be investigated as to its anti-allergy activity for support of using Thai traditional medicine.

Prabchompoothaweep remedy (PT) is composed of 23 medicinal plants. The ratio of plants ingredients in PT remedy is in the National herbal drug list of Thailand and ethnopharmaceutical used is shown in Table 1. There is no report on the anti-allergic activity of PT remedy extract and related activities with allergy such as anti-inflammatory and antioxidant activity. Thus, the objective of this research was to study on anti-allergy, anti-inflammatory and antioxidant activity of PT extracts by different extraction method. This result will be guided for develop PT product for allergic diseases.

Material and Method

Chemicals and reagents

MEM medium, RPMI medium 1640, fetal bovine serum (FBS) came from Gibco (NY, USA), Phosphate buffered saline (PBS) from Amresco (Ohio, USA), Sodium bicarbonate from BDH (Poole, England), Penicillin-Streptomycin (P/S), trypsin-EDTA; lipopolysaccharide from *E. coli* (LPS), 3-(4,5-dimethyl-2-thiazolyl)-2, 5-diphenyl-2 *H*-tetrazolium bromide (MTT), *N*-(1-naphthyl) ethylenediamine dihydrochloride, phosphoric acid solution, sulfanilamide, anti-dinitrophenylated bovine albumin (DNP-BSA), anti-DNP IgE (Monoclonal Anti-DNP) and 4-Nitrophenyl N-acetyl-β-D-glucosaminide (PNAG) from Sigma-Aldrich Inc. (MO, USA). Hydrochloric acid fuming 37% came from Merck (Darmstadt, Germany), Dimethylsulfoxide (DMSO) and isopropanol from RCI Labscan Limited (Bangkok, Thailand), Butylated hydroxytoluene (BHT) and 2,2-Diphenyl-2-picrylhydrazyl (DPPH) from Fluka (Seelze, Germany).

Cell culture

Anti-allergic activity

Rat basophilic leukemia cell lines (RBL-2H3) was purchased from the American type culture collection (ATCC) (CRL-2256, VA, USA). The cells were cultured in minimum essential medium eagle (MEM) supplemented with 15% heat-inactivated fetal bovine serum (FBS), 2 mM glutamine, 100 U/ml penicillin and 100 µg/ml streptomycin, and maintained at 37°C in 5% CO_2.

Anti-inflammatory activity

Murine macrophage-leukemia cells lines (RAW 264.7) [American type culture collection (ATCC) (TIB-71, VA, USA)] were cultured in RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum (FBS), 2 mM glutamine, 100 U/ml penicillin and 100 µg/ml streptomycin, and maintained at 37°C in 5% CO_2.

Plant materials and extraction

Plant materials were purchased from Charernsook osot pharmacy (Nakornpathom, Thailand) and showed in Table 1. The plants were cleaned, dried at 45°C in an oven. Dried plant materials were ground to be powder (Mesh size 40). Each plant materials was weighed in the ratio of PT following in National herbal drug list of Thailand and mixed as PT remedy. The extraction methods were maceration and decoction.

Maceration method

The crude powder of PT (500 g) were macerated in 95% ethanol (2L) and 50% ethanol (2L) for 3 days and filtered. The extraction was continuously re-extracted two times and dried by using a rotary evaporator. The extracts were dried to constant weight in a vacuum dryer at 40°C. The extracts obtained to be PTE95 and PTE50. The residues after maceration were continuously extracted by decoction method. The residues after maceration were boiled in distilled water (3L) at boiling point for 15 min and filtered. The residue was further boiled again two times. The combined water extracts were evaporated to dryness using a lyophillizer. The extracts from residue obtained to be PTR95 and PTR50.

Decoction method

The crude powder of PT (500 g) was boiled in distilled water (3L) for 15 min and filtered. The residue was further boiled again two times. The combined water extracts were evaporated to dryness by using a lyophilizer. This extract obtained to be PTW.

The crude extracts were calculated percentage of yield and stored in airtight glass container at -20°C before use. The percentage yield (% w/w) of the plant extracts are shown in Table 2.

Table 1. The ethnobotanical data of Prabchompoothaweep ingredients

Species	Family	Voucher specimen number	Thai name	Part used	Percent in remedy	Traditional used*
Acanthus ebracteatus Vahl.	ACANTHACEAE	SKP001010501	Ngueakplamo	Arial part	25.81	Promote lymphatic system, Anti dermatitis
Anethum grareolens L.	UMBELLIFERAE	SKP199010701	Thian tatakkataen	Seed	1.29	Carminative
Angelica dahurica Benth.	UMBELLIFERAE	SKP199011901	Kot sor	Rhizome	0.86	Tonic, Antipyretic, Paregoric, Diuretic Agents, Relieve common cold, Relieve asthma, Oral prophylaxis
Amomum testaceum Ridl.	ZINGIBERACEAE	SKP206011101	Krawan	Fruit	0.22	Promote blood circulation, Carminative
Amorphophallus saraburiensis (Dennst)	ARACEAE	SKP015011901	Book ror	Rhizome	1.72	Promote lymphatic system
Ardisia elliptica Thunb.	MYRSINACEAE	SKP122011601	Philangkasa	Fruit	0.86	Anti-diarrhe, urticaria
Atractylodes lancea (Thunb) D.C.	COMPOSITAE	SKP051011201	Kot khamoa	Rhizome	0.89	Antipyretic, Tonic, Relieve cough and asthma
Cinnamomum camphora (L.) Presl	LAURACEAE	SKP096030301	Karabool	Camphor	0.43	Cardio tonic, Diuretic Agents, Relieve Myalgia
Clausena excavate Burm f	RUTACEAE	SKP183110801	Hasakhun thet	Stem	2.15	Promote blood circulation, Anti nasal polyp
Entalus acoroids (Lf) Rovle	HYDROCARITACEAE	SKP088050101	Lampan hangmoo	Rhizome	0.86	Promote lymphatic system
Foeniculum vulgare Mill	UMBELLIFERAE	SKP199062201	Thian klaeb	Seed	1.29	Carminative
Leonurus sibiricus L	LAMIACEAE	SKP095121901	Kancha thet	Arial part	25.81	Promote blood circulation, Antipyretic, Gynecology, Diuretic Agents Anti-inflammatory
Lepidium sativum L	CRUCIFERAE	SKP019121901	Thien dang	Seed	1.29	Gastrointestinal agents, Carminative
Myristica fragrans Houtt	MYRISTICACEAE	SKP121130601	Dok chan	Mace	0.22	Tonic, Cardio tonic, Carminative
Myristica fragrans Houtt	MYRISTICACEAE	SKP121130601	Look chan	Nutmeg	0.22	Tonic, Cardio tonic, Carminative
Nigella sativa L	RANUNCULACEAE	SKP160141901	Thian dam	Seed	0.86	Carminative, Antiemetic
Plumbago indica L	PLUMBAGINACEAE	SKP148160901	Chetamoolplerng	Root	1.72	Promote blood circulation, Gynecology, Carminative
Piper nigrum L	PIPERACEAE	SKP146161401	Prikthai dam	Seed	25.81	Promote blood circulation, Carminative, Tonic for longevity
Piper retrofraotum Vahl	PIPERACEAE	SKP146161801	Deeplee	Flower	0.43	Promote blood circulation, Carminative, Relieve asthma, Reduce phlegm, Relieve Myalgia, Uterine stimulant
Syzygium aromaticum (L) Merr et Perry	MYRTACEAE	SKP123190101	Kan phlu	Flower	2.15	Carminative, Paregoric of tooth and pyorrhea, Promote blood circulation
Terminalia sp.	COMBRETACEAE	SKP049200101	Samor thet	Fruit	1.72	Laxative, Anti-diarrhea, Relieve cough
Terminalia chebula Rez var Chebula	COMBRETACEAE	SKP049200301	Samor thai	Fruit	1.72	Laxative, Anti-diarrhea, Relieve cough
Zingiber officinale Roscoe	ZINGIBERACEAE	SKP206261501	Khing	Rhizome	1.72	Promote blood circulation, Carminative, Reduce phlegm, Antiemetic

* Thai traditional used are reviewed from the explanation text of Osot Pra Narai[25] and encyclopedia and principle of Thai traditional pharmacy[26]

Table 2. Percentage of yield, percent inhibition at various concentrations and IC_{50} values of PT extracts on the release of β-hexosamonidase from RBL-2H3 cells (n = 3)

Extract	Yield (% w/w)	Inhibition (%) at various concentrations (μg/ml)				IC_{50} ± SEM (μg/ml)
		1	10	50	100	
PTE95	12.08	20.60±5.60	44.07±3.20	64.45±0.63	77.91±4.35	12.97±1.92
PTE50	19.25	-2.53±6.62	9.80±3.55	43.52±1.00	58.86±0.55	65.36±2.46
PTR95	28.06	-	-	-9.48±0.51	-9.10±0.64	>100
PTR50	27.75	-	-	-14.20±0.70	-15.36±0.46	>100
PTW	26.84	-	-	-7.65±3.00	1.08±0.80	>100
CPM	-	1.98±2.55	26.61±2.24	72.80±0.93	95.14±1.19	22.16±1.07

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

The inhibition of PT extracts on the release of β-hexosaminidase from RBL-2H3 cell lines was modified method from Tewtrakul and Itharat (2006). Briefly, RBL-2H3 cells ($2x10^5$ cells/well) were seeded in 24-well plates and allowed to adhere for 1 hour at 37°C in 5% CO_2. The cells were sensitized with 0.45 μg/ml anti-DNP-IgE overnight at 37°C in 5% CO_2. The cells were washed with 400 μl of Siraganian buffer [buffer A, containing 119 mM NaCl, 5 mM KCl, 5.6 mM D-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 minutes at 37°C in 5% CO_2. After that, 20 μl of different concentrations of the samples were added to each well and incubated at 37°C in 5% CO_2 for 10 minutes, and then 20 μl of DNP-BSA (10 μg/ml) was added and incubated at 37°C in 5% CO_2 for 20 minutes to stimulate the cells to degranulate. Aliquots (50 μl) of supernatant was then transferred to 96-well plates and incubated with 50 μl of PNAG in 0.1 M citrate buffer (pH 4.5) at 37°C in 5% CO_2 for 1 hour. The reaction was stopped by adding 200 μl of stop solution (0.1 M Na_2CO_3/$NaHCO_3$, pH 10.0). Chlorpheniramine (CPM), an anti-allergic drug, was used reference standard. The absorbance was measured at 405 nm using a microplate reader. The inhibition (%) of the release of β-hexosaminidase by the sample was calculated by the following equation, an IC_{50} values were graphically determined.

$$\% \text{ inhibition} = \frac{1-(T-B-N)}{(C-N)} \times 100$$

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 (+).

Inhibitory effects on the release of NO from RAW 264.7 cell lines

The inhibition of NO production from RAW 264.7 cell lines was modified method from Tewtrakul and Itharat (2007). Briefly, RAW 264.7 was cultured in 96-well microplate with 100 μl complete RPMI ($1x10^5$ cells/well) and incubated in 5% CO_2 at 37°C for 24 hours. Complete RPMI (100 μl/well) contained 10 ng/ml of lipopolysaccharide (LPS) and was replaced in control and complete RPMI without LPS (100 μl/well) which was replaced as normal. The stock samples (50 mg/ml) were initially dissolved in DMSO for ethanolic extracts, which were applied at a final concentration of 0.2% (v/v) in cell culture supernatants, or dissolved in sterile distilled water, for water extracts. After that, the cells were tested with samples (100 μl/well) and incubated for 24 hours. Next, 100 μl of supernatant was transferred to the 96-well microplate and mixed with 100 μl of Griess reagent (0.1% *N*-(1-Naphthyl) ethylenediamine dihydrochloride and 1% sulfanilamide in 2.5% H_3PO_4). The NO production was determined by measuring the accumulation of nitrite which interacted with Griess reagent. Cytotoxicity was also determined by the MTT colorimetric assay. Briefly, after 24 hours incubation with test sample, MTT solution (10 μl, 5 mg/ml in PBS) was added to the wells. After a 2-hour incubation, the medium was removed, and isopropanol containing 0.04 M HCl was added to dissolve the formazan production in the cells. The absorbance was measured at 570 nm using a microplate reader. The test sample was considered cytotoxic when the optical density of the sample-treated group was less than 70% of that in the control group. Prednisolone was used as reference

Table 3. The inhibition (%) at various concentrations and IC_{50} values of PT extracts on the LPS-induced of NO production from RAW 264.7 cells (n = 3)

Extract	Inhibition (%) at various concentrations/(cytotoxicity (%) at various concentrations) (µg/ml)						IC_{50} ± SEM (µg/ml)
	0.01	0.1	1	10	50	100	
PTE95	-	-	12.46±1.48 (11.51±6.56)	29.30±0.86 (-6.92±11.17)	81.55±2.45 (-1.73±6.27)	96.56±0.35 (4.62±10.12)	22.51±0.68
PTE50	-	-	1.13±1.55 (-5.32±5.68)	16.77±2.2 (-2.85±1.20)	44.57±0.90 (-18.92±10.18)	73.65±2.14 (-18.18±5.30)	61.96±1.92
PTR95	-	-	7.60±1.83 (-5.76±4.66)	6.73±3.27 (-11.97±6.17)	55.04±3.12 (-1.22±5.20)	80.68±4.62 (10.82±4.30)	46.23±0.53
PTR50	-	-	-	-	64.06±12.11 (41.24±3.60)	77.70±1.48 (52.98±0.70)	Toxic
PTW	-	-	-	-	31.11±3.85 (-23.47±2.37)	47.94±2.08 (-0.52±0.25)	>100
Prednisolone	6.90±5.69 (2.45±4.35)	30.38±3.90 (17.80±3.52)	44.62±0.61 (24.95±2.66)	54.13±2.15 (28.58±0.97)	-	-	0.21±0.15

standard. The inhibition (%) of the release of NO by the sample was calculated by the following equation, an IC_{50} values were graphically determined.

$$\% \text{ inhibition} = \frac{A-B}{A-C} \times 100$$

A-C was NO_2^- concentration (µM) [A was LPS (+) and test sample (-); B was LPS (+) and test sample (+); C was LPS (-) and test sample (-)]

DPPH radical scavenging assay

Antioxidant activity of extracts on DPPH radical scavenging assay was a modified method from Yamazaki (1994). Sample for testing was dissolved in absolute ethanol or distilled water at various concentrations. A portion of the sample solution (100 µl) was mixed with an equal volume of $6x10^{-5}$ M DPPH (in absolute ethanol) and allowed to stand in the dark at room temperature for 30 minutes. The absorbance (OD) was measured at 520 nm using a microplate reader. BHT, a well known synthetic antioxidant, was tested in the same system as a reference standard. The scavenging activity of the sample corresponded to the reduction in the intensity of DPPH. The inhibition (%) was calculated by the following equation, an EC_{50} values were graphically determined.

$$\% \text{ inhibition} = [(OD_{control}-OD_{sample})/OD_{control}] \times 100$$

Statistical analysis

The results were expressed as the mean ± SEM of three determinations at each concentration for each sample. The IC_{50} and EC_{50} values were calculated using the prism program.

Results

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

The inhibitory effects of PT extracts on IgE-induced degranulation in sensitized RBL-2H3 cell lines were evaluated. β-hexosaminidase was used as a marker of the degranulation of RBL-2H3 cell lines. The results shown in Table 2, PTE95 exhibited the most potent inhibitory activity with an IC_{50} value of 12.97±1.92 µg/ml, followed by PTE50 with an IC_{50} value of 65.36±2.46 µg/ml. On the other hand, PTR95, PTR50 and PTW had no measurable activity (IC_{50} >100 µg/ml). The anti-allergic activity of PTE95 was higher than that of reference standard, CPM (IC_{50} = 22.16±1.07 µg/ml, 80.64 µM), whereas that of PTE50 was lower than the reference standard.

Table 4. The inhibition (%) at various concentrations and EC_{50} values of PT extracts on DPPH radical scavenging activity (n = 3)

Extract	Inhibition (%) at various concentrations (µg/ml)				EC_{50} ± SEM (µg/ml)
	1	10	50	100	
PTE95	7.60±1.64	37.36±3.75	92.78±1.87	94.92±0.75	14.62±1.13
PTE50	5.74±1.32	35.30±1.27	80.20±2.80	91.47±1.13	15.83±0.96
PTR95	3.84±4.21	26.30±1.01	70.17±1.00	85.79±3.73	23.56±1.68
PTR50	1.77±0.17	12.64±2.22	45.45±1.00	68.84±1.97	58.12±0.97
PTW	-0.10±1.58	23.90±1.69	72.70±0.59	85.63±0.97	23.72±1.02
BHT	5.37±0.51	37.67±2.36	76.80±0.34	84.03±1.20	14.49±1.22

Inhibitory effects on the release of NO from RAW 264.7 cell lines

The inhibitory effects of PT extracts on LPS-induced NO production in RAW 264.7 cell lines were evaluated. The results shown in Table 3, PTE95 also presented the highest potent activity with an IC_{50} value of 22.51±0.53 µg/ml, followed by PTR95 (IC_{50} = 46.23±0.68 µg/ml) and PTE50 (IC_{50} = 61.96±1.92 µg/ml). On the other hand, PTW had no measurable activity (IC_{50} >100 µg/ml) and PTR50 showed high cytotoxicity (% cytotoxicity more than 30). However, all the extracts that of measurable activity were lower than Prednisolone as the reference standard (IC_{50} = 0.21±0.15 µg/ml, 0.58 µM).

DPPH radical scavenging assay

Antioxidant activity of PT extracts on DPPH radical scavenging assay was evaluated. The results shown in Table 4, PTE95 also exhibited the most potent antioxidant activity with an EC_{50} value of 14.62±1.13 µg/ml, followed by PTE50, PTR95, PTW and PTR50 (EC_{50} = 15.83±0.96, 23.56±1.02, 23.72±1.68 and 58.12±0.97 µg/ml, respectively). In addition, all extracts had less antioxidant activity than BHT as the reference standard (EC_{50} = 14.49±1.22 µg/ml).

Discussion

PTE95 had stronger anti-allergic activity than CPM as reference standard. The extraction method for preparing PT products should be extracted by maceration in 95% ethanol. Polar solvent such as water and 50% ethanol lead to the extract having less activity. It is possible that PT composed with two species of *Piper* with high rich piperine and *Piper nigrum* was in PT at 25.81%. This study is comparable with the previous report. *P. nigrum* and its compound (piperine) showed strong anti-allergic activity[13]. Interestingly, the activity of PTE95 related to *P. nigrum* as one of the highest ingredients in PT remedy. In addition the aqueous extract of *S. aromaticum* was studied in animal models; it inhibited compound 48/80-induced systemic anaphylaxis (IC_{50} = 31.25 mg/kg, ip), local immunogloburin E (IgE)-mediated passive cutaneous anaphylactic reaction (IC_{50} = 17.78 mg/kg, iv, 19.81 mg/kg, po)[14].

Inflammation is a part of biological responses of our body to protect against pathogens, physical and chemical stimuli. The immune cells were involve in the inflammatory process as well as macrophage directly triggering immunological events such as release of cytokines, chemokines and inflammatory-mediators[15]. NO is a one of the inflammatory mediators, it is causing inflammation on many organs[16]. PTE95 had the most potent anti-inflammatory activity via LPS-induced NO production. PTR95 and PTE50 showed the moderately potent activity. However, PTE95 shows less anti-inflammatory activity than prednisolone as 107.2 times. In a recent report on *P. nigrum* and *L. sibiricus*, which are the highest ingredients in PT, were shown to have strong anti-inflammatory activity[17,18]. In vitro study, its ingredients of PT have reported that ethanolic extract of *T. chebula*, *A. lancea*, *P. retrofraotum*, *A. dahurica*, *C. excavate*, *S. aromaticum* and *A. testaceum* inhibited LPS-induced NO production in RAW 264.7 cells (IC_{50} = 3.30, 9.70, 25.90, 44.23, 74.70, 81.42 and 81.34 µg/ml, respectively)[19,20]. In addition, Atractylenolide I was isolated from the rhizome *A. lancea* that inhibited LPS-induced NO production with IC_{50} value of 7.5 µg/ml[21].

For DPPH radical scavenging activity, PTE95 also showed the highest antioxidant activity, However, PTE50, PTR95 and PTW gave high antioxidant power.

The ingredients of PT have often been reported. For example, the macelignan isolated from *M. fragrans* seed possesses the strong DPPH free radical scavenging activity (IC_{50} = 25 μM)[22]. The clove bud extract, eugenol, eugenyl acetate and benzyl alcohol inhibited malonaldelhyde formation from cod liver oil by 93, 88, 79 and 63%, respectively, at the level of 160 μg/ml[23]. The ethanolic extract of *T. chebula* exhibited strong DPPH radical scavenging assay (EC_{50} = 5.0 μg/ml)[20] because *T. chebula* extract presents high phenolic compounds[24].

From all PT activity, it could be concluded that the method, which gave the best activity to treat allergic rhinitis or allergic diseases, is maceration with 95% ethanol. By this reasoning, this part gave the highest anti-allergy, anti-inflammatory and antioxidant activities. Interestingly, PT looks like a combination of western medicine. Normally, the drug of choice for allergy treatment is an anti-allergy and anti-inflammatory drug. Thus, PT has two activities for allergic treatment. In addition, PT also has antioxidant activity, which reduces causes of increasing inflammation and allergy and helps to increase (enhance) immune system in body.

Conclusion

PTE95 showed the highest anti-allergic, anti-inflammatory and antioxidant effects. These results suggest that the best of extraction method for PT is maceration method that gave the highest related activity with allergy. The crude ethanolic extract of PT should be developed as anti-allergic drug for allergic rhinitis treatment. These results can support using PT for treatment of allergic rhinitis and asthma based on Thai traditional medicine and the National herbal drug list of Thailand. However, further studies are necessary to test crude 95% ethanolic extract from plant ingredients to find the active plant ingredient and isolate active ingredients from the ethanolic extract of PT. The active ingredient should be a marker for analysis of PT remedy.

Acknowledgement

The authors wish to thank the National Research University Project of Thailand Office of Higher Education Commission, Center of Excellence on Applied Thai Traditional Medicine Research (CEATMR) and Thammasat University, Thailand, for financial support.

Potential conflicts of interest

None.

References

1. Nakatani K, Atsumi M, Arakawa T, Oosawa K, Shimura S, Nakahata N, et al. Inhibitions of histamine release and prostaglandin E2 synthesis by mangosteen, a Thai medicinal plant. Biol Pharm Bull 2002; 25: 1137-41.
2. Pawankar R, Canonica GW, Holgate ST, Lockey RF. Introduction to the executive summary. In: WAO white book on allergy 2011-2012: executive summary. Milwaukee, WI: World Allergy Organization; 2011: 1-2.
3. Salib RJ, Drake-Lee A, Howarth PH. Allergic rhinitis: past, present and the future. Clin Otolaryngol Allied Sci 2003; 28: 291-303.
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. Downie SR, Andersson M, Rimmer J, Leuppi JD, Xuan W, Akerlund A, et al. Association between nasal and bronchial symptoms in subjects with persistent allergic rhinitis. Allergy 2004; 59: 320-6.
6. Goldsby RA, Kindt TJ, Osborne BA, Kuby J. Kuby Immunology. 5th ed. New York: W.H. Freeman; 2003.
7. Schwartz LB, Austen KF. Enzymes of the mast cell granule. J Invest Dermatol 1980; 74: 349-53.
8. Guo YC, Li ZX, Lin H. Investigation on the relationship among histamine, tryptase and beta-hexosaminidase in the process of mast cell degranulation. Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi 2009; 25: 1073-5.
9. Department for Development of Thai Traditional and Alternative Medicine. List of herbal medicine products A.D 2011. 2nd ed. Bangkok: The War Veterans Organization of Thailand Under Royal Patronage of His Majesty the King; 2011.
10. Tewtrakul S, Itharat A. Anti-allergic substances from the rhizomes of *Dioscorea membranacea*. Bioorg Med Chem 2006; 14: 8707-11.
11. Tewtrakul S, Itharat A. Nitric oxide inhibitory substances from the rhizomes of *Dioscorea membranacea*. J Ethnopharmacol 2007; 109: 412-6.
12. Yamasaki K, Hashimoto A, Kokusenya Y, Miyamoto T, Sato T. Electrochemical method for estimating the antioxidative effects of methanol extracts of crude drugs. Chem Pharm Bull (Tokyo) 1994; 42:

1663-5.
13. Kraithep S, Oungbho K, Tewtrakul S. Anti-allergic activity of Thai medicinal plants used in longevity formulation. Songklanakarin J Sci Technol 2008; 30: 621-5.
14. Kim HM, Lee EH, Hong SH, Song HJ, Shin MK, Kim SH, et al. Effect of *Syzygium aromaticum* extract on immediate hypersensitivity in rats. J Ethnopharmacol 1998; 60: 125-31.
15. Burodom A, Itharat A. Inflammatory suppressive effect of Benjakul, a Thai traditional medicine on intestinal epithelial cell line. J Med Plants Res 2013; 7: 3286-91.
16. Hetrick EM, Schoenfisch MH. Analytical chemistry of nitric oxide. Annu Rev Anal Chem (Palo Alto Calif) 2009; 2: 409-33.
17. Ahmad N, Fazal H, Abbasi BH, Ferooq S, Ali M, Khan MA. Biological role of *Piper nigrum* L. (Black pepper): A review. Asian Pac J Trop Biomed 2012; 2: S1945-53.
18. Shin HY, Kim SH, Kang SM, Chang IJ, Kim SY, Jeon H, et al. Anti-inflammatory activity of Motherwort (*Leonurus sibiricus* L). Immunopharmacol Immunotoxicol 2009; 31: 209-13.
19. Makchuchit S, Itharat A, Tewtrakul S. Antioxidant and nitric oxide inhibition activities of Thai medicinal plants. J Med Assoc Thai 2010; 93 (Suppl 7): S227-35.
20. Kakatum N, Jaiarree N, Makchucit S, Itharat A. Antioxidant and anti-inflammatory activities of Thai medicinal plants in Sahasthara remedy for muscle pain treatment. J Med Assoc Thai 2012; 95 (Suppl 1): S120-6.
21. Wang C, He L, Wang N, Liu F. Screening anti-inflammatory components from Chinese traditional medicines using a peritoneal macrophage/cell membrane chromatography-offline-GC/MS method. J Chromatogr B Analyt Technol Biomed Life Sci 2009; 877: 3019-24.
22. Jin DQ, Lim CS, Hwang JK, Ha I, Han JS. Anti-oxidant and anti-inflammatory activities of macelignan in murine hippocampal cell line and primary culture of rat microglial cells. Biochem Biophys Res Commun 2005; 331: 1264-9.
23. Lee KG, Shibamoto T. Antioxidant property of aroma extract isolated from clove buds [*Syzygium aromaticum* (L.) Merr. et Perry]. Food Chem 2001; 74: 443-8.
24. Manosroi A, Jantrawut P, Akazawa H, Akihisa T, Manosroi J. Biological activities of phenolic compounds isolated from galls of *Terminalia chebula* Retz. (Combretaceae). Nat Prod Res 2010; 24: 1915-26.
25. Phichianshunthorn C, Chawalit M, Jeerawong W. Explanation the text of Osot Pra Narai. Bangkok: Amarin Printing and Publishing; 2005.
26. Whuthidhamawet W. Encyclopedia and principle of Thai traditional pharmacy. Bangkok: Odeon Store; 1997.

ฤทธิ์ต้านการแพ้ฤทธิ์ต้านการอักเสบและฤทธิ์ต้านอนุมูลอิสระของสารสกัดตำรับปราบชมพูทวีปที่ใช้รักษาโรคจมูกอักเสบจากภูมิแพ้

อารีรัตน์ ใจเอื้อ, ศุณิตา มากชูชิต, ธนา จักษ์เมธา, อรุณพร อิฐรัตน์

ภูมิหลัง: *ปราบชมพูทวีปเป็นตำรายาไทยใช้บรรเทาอาการโรคจมูกอักเสบภูมิแพ้และหอบหืด ที่ประกอบไปด้วยสมุนไพร 23 ชนิด อยู่ในบัญชียาหลักแห่งชาติ ซึ่งไม่เคยมีรายงานการศึกษาถึงฤทธิ์ต้านการแพ้ ฤทธิ์ต้านการอักเสบ และฤทธิ์ต้านอนุมูลอิสระมาก่อน*

วัตถุประสงค์: *เพื่อศึกษาฤทธิ์ต้านการแพ้ ฤทธิ์ต้านการอักเสบ และฤทธิ์ต้านอนุมูลอิสระของสารสกัดตำรับยาปราบชมพูทวีปด้วยวิธีการสกัดที่ต่างกัน*

วัสดุและวิธีการ: *วิธีการสกัด สกัดด้วยการหมักเอทานอล 95% หมักเอทานอล 50% หลังจากนั้นนำกากที่เหลือจากการสกัดด้วยการหมักเอทนอลมาต้มน้ำ ได้สารสกัด PTE95 PTE50 PTR95 และ PTR50 วิธีการสกัดอีกวิธีคือ สกัดด้วยการต้มน้ำและทำให้แห้งด้วยเครื่องระเหิดแห้งได้สารสกัด PTW สารสกัดทั้ง 5 สารสกัดนำไปทดสอบฤทธิ์ต้านการแพ้โดยดูการยับยั้งเอนไซม์ beta-hexosaminidase จากเซลล์ RBL-2H3 ทดสอบฤทธิ์ต้านการอักเสบโดยดูการยั้บยั้งการสร้างไนตริกออกไซด์จากเซลล์ RAW 264.7 และทดสอบฤทธิ์ต้านอนุมูลอิสระโดยวิธี DPPH radical scavenging assay*

ผลการศึกษา: *สารสกัด PTE95 ให้ผลการทดสอบดีที่สุดไม่ว่าจะเป็นฤทธิ์การต้านการแพ้ (ค่า IC_{50} เท่ากับ 12.97 ไมโครกรัมต่อมิลลิลิตร) ฤทธิ์ต้านการอักเสบ (ค่า IC_{50} เท่ากับ 22.51 ไมโครกรัมต่อมิลลิลิตร) และฤทธิ์ต้านอนุมูลอิสระ (ค่า EC_{50} เท่ากับ 14.62 ไมโครกรัมต่อมิลลิลิตร)*

สรุป: *สารสกัดตำรับยาปราบชมพูทวีปชั้นเอทานอลสามารถต้านการเกิดการแพ้ ต้านการหลั่งไนตริกออกไซดใช้เป็นข้อมูลสนับสนุนการใช้ยาตำรับนี้ในการรักษาโรคจมูกอักเสบจากภูมิแพ้และหอบหืดบนพื้นฐานของการแพทย์แผนไทยได้*