Songklanakarin J. Sci. Technol.
30 (4), 467-473, Jul. - Aug. 2008

Songklanakarin Journal of Science and Technology
http://www.sjst.psu.ac.th

Short Communication

Anti-allergic and anti-microbial activities of some Thai crops

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Received 29 May 2008; Accepted 10 July 2008

Abstract

Thirteen Thai crops including banana, okra, jackfruit, germinated rice, rambutan, durian, jampadah, huasa potato, tamarind, coconut, mango, fan palm fruit and dioscorea tuber were tested for anti-allergic effect using RBL-2H3 cells and anti-microbial activity. These 13 crops, some of which included different parts, e.g. skin, flesh, and seed, were extracted with four solvents separately [(95% ethanol (EtOH), 50% EtOH, water (W) and hot water (HW)], respectively, to obtain 112 extracts. Among these extracts, mango seed in 50% EtOH possessed the highest anti-allergic activity against antigen-induced β-hexosaminidase release as a marker of degranulation in RBL-2H3 cells with an IC_{50} value of 7.5±0.8 μg/ml, followed by banana (W, IC_{50} = 13.5±2.4 μg/ml), okra (W, IC_{50} = 13.6±3.1 μg/ml), jampadah skin (HW, IC_{50} = 13.8±3.9 μg/ml), tamarind seed coat (HW, IC_{50} = 14.2±3.1 μg/ml), jampadah flesh (W, IC_{50} = 14.6±3.1 μg/ml); whereas other crops possessed IC_{50} values from 21.5->100 μg/ml. Moreover, the plants showing high anti-allergic effects were also possessed marked anti-bacterial activity. Rambutan peel, mango peel, mango seed and tamarind seed coat exhibited appropriate anti-bacterial activity against *Staphylococcus aureus*, *Bacillus subtilis* and *Pseudomonas aeruginosa* with MIC values ranging from 250-2,000 μg/ml, but did not show any effect towards *Escherichia coli* and *Candida albicans*. This study indicates that these Thai crops may have potential as functional foods and neutraceuticals for treatment of allergy, allergy-related diseases and some bacterial infections.

Keywords: RBL-2H3 cells, anti-allergic activity, anti-microbial activity, Thai crops

1. Introduction

Allergy is an immune dysfunction, which is a serious health problem worldwide. Substances that cause allergic reaction are called allergens and include food, pollen, dust mites, cosmetics, mold spores and animal hairs. Hypersensitivity type I, an allergic reaction, is an IgE-mediated immune response, resulting in histamine secretion from mast cells and blood basophils. The histamine causes smooth muscle contraction, increased vascular permeability and vasodilation. The early phase reaction of allergy occurs within minutes after allergen exposure, whereas the late phase reaction occurs hours later and involves cytokines secretion such as TNF-α and IL-4 (Goldsby *et al.*, 2002). Since β-hexosaminidase is usually released along with histamine from mast cells or basophils, this enzyme is used as the marker for mast cell degranulation in RBL-2H3 cell line (Cheong *et al.*, 1998).

Nutraceutical and functional food (NFF) market is worth more than $500 billion annually on a global scale. There is a wide range of NFF products designed for specific health purposes including sports or energy drinks, high fibre

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products, vitamin or mineral fortified foods and other health-related products. In the present study, 13 Thai crops that are locally grown in the southern part of Thailand, including banana (*Musa sapientum* Linn.), okra (*Hibiscus esculentus* Linn.), jackfruit (*Artocarpus heterophyllus* Lamk.), germinated rice (*Oryza sativa* Linn.), rambutan (*Nepheleum lappaceum* Linn.), durian (*Durio zibethinus* Linn.), jampadah (*Artocarpus integer* Merr.), huasa potato (*Coleus parvifolius* Benth), tamarind (*Tamarindus indica* Linn.), coconut (*Cocos nucifera* Linn.), mango (*Mangifera indica* Linn.), fan palm fruit (*Borassus flabellifer* Linn.) and dioscorea tuber (*Dioscorea membranacea* Pierre) were tested for anti-allergic effect using RBL-2H3 cells and anti-microbial effects against *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Candida albicans.*

2. Materials and Methods

2.1 Reagents

Minimum Essential Medium Eagle (MEM) and anti-DNP-IgE (Monoclonal anti-DNP) were purchased from Sigma; fetal calf serum (FCS) was from Gibco; dinitrophenylated bovine serum albumin was prepared as described previously (Tada and Okumura, 1971). Other chemicals were from Sigma. 24-well and 96-well plates were from Nunc.

2.2 Plant materials

Thirteen crops locally available in southern Thailand, including banana (SKP119131901), okra (SKP109080501), jackfruit (SKP117010801), rambutan (SKP171141201), durian (SKP027042601), jampadah (SKP117010901), huasa potato (SKP095031601), tamarind (SKP034200901), coconut (SKP136031401), mango (SKP009130901), fan palm fruit (SKP1360120601) and dioscorea tuber (SKP062041301) were bought from the market in Songkhla province, Thailand. Germinated rice (SKP081151901) was supplied by Department of Agro-Industry, Naresuan University, Thailand. The numbers in parenthesis are voucher specimen numbers.

2.3 Preparation of the plant extracts

Five hundreds grams dried weight of each crop was ground and macerated with 95% EtOH, 50% EtOH, water and hot water, separately 3 times (1 L x 3) at room temperature. The extracts were evaporated under vacuum and freeze dried. The freeze-dried extracts were kept at -20°C before use.

2.4 Anti-allergic activity assay

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

Inhibitory effects on the release of β-hexosaminidase from RBL-2H3 cells (purchased from ATCC, USA) were evaluated by the following modified method (Matsuda *et al.*, 2004). Briefly, RBL-2H3 cells were dispensed in 24-well plates at a concentration of 2×10^5 cells/well using Minimum Essential Medium Eagle (MEM) containing 10% fetal calf serum (FCS), penicillin (100 units/ml), streptomycin (100 unit/ml) and anti-dinitrophenyl-immunoglobulin E (anti-DNP IgE) (0.45 μg/ml), then incubated overnight at 37°C in 5% CO_2 for sensitization of the cells. The cells were washed twice with 500 μl of Siraganian buffer [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 an additional 10 min at 37°C. Subsequently, 20 μl of test sample solution was added to each well and incubated for 10 min, followed by the addition of 20 μl of antigen (DNP-BSA, final concentration is 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 (1mM *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 test sample was dissolved in dimethylsulfoxide (DMSO), and the solution was added to Siraganian buffer (final DMSO concentration was 0.1 %). Ketotifen fumarate, a clinical used drug, was used as a positive control. The inhibition (%) of the release of β-hexosaminidase by the test samples was calculated by the equation shown below, and the samples whose activity at 100 μg/ml was more than 80% inhibition, were further evaluated for IC_{50} values. The IC_{50} values were determined graphically:

$$\text{Inhibition } \% = [1- (T-B-N)/(C-N)] \times 100$$

Control (C): DNP-BSA (+), Test sample (-); Test (T) : DNP-BSA (+), Test sample (+); Blank (B) : DNP-BSA (-), Test sample (+); Normal (N) : DNP-BSA (-), Test sample (-)

2.5 Anti-microbial activity assay

1) Microorganisms

The tested organisms used in the study included two Gram-positive bacteria: *S. aureus* ATCC 25923 and *B. subtilis*; two Gram-negative bacteria: *E. coli* ATCC 25922 and *P. aeruginosa* which were kindly provided by Department of Pathology, Faculty of Medicine, Prince of Songkla University, Thailand. Fungus used was *C. albicans* TISTR 5779 which was provided by Department of Microbiology, Faculty of Sciences, Prince of Songkla University, Thailand.

2) Media

The medium used in the assay for the anti-bacterial

test was Mueller Hinton agar (MHA, Merck, Germany) and that for the anti-fungal test was Sabouraud Dextrose agar (SDA, Merck, Germany).

3) Reference antibiotics

Tetracycline (Oxoid, Oxoid Limited, England) and norfloxacin (Oxoid, Oxoid Limited, England) were used as reference antibiotics.

4) Preliminary susceptibility test

The disc diffusion method (Lorian, 1996) was used to screen the antimicrobial activity. Sterile disks (6 mm) were impregnated with 10 µl of crude extract at the concentration of 100 mg/ml. For bacteria, the microbes were placed on the surface of Mueller Hinton agar (MHA), whereas Sabouraud dextrose agar (SDA) was used for fungi. The extract was tested in triplicate; control discs contained 10 µl of dimethyl sulfoxide (DMSO) and distilled water. The standard antibiotics consisted of tetracycline (30 µg/disc) for the Gram-positive bacteria, norfloxacin (10 µg/disc) for the Gram-negative bacteria. The plates were incubated at 37°C for 24 h and 48 h for the bacteria and yeast, respectively.

5) Determination of minimum inhibitory concentration (MIC)

The extracts showing marked inhibition zones were further studied to determine their MIC values. The MIC values were calculated by the microdilution method according to the reported literature (Lorian, 1996). The bacteria were grown on MHA for 24 h. The inocula (2 µl) containing 10^4 cfu of each microorganism were spotted on agar supplemented with an extract or antibiotic at concentrations ranging from 2,000-15.62 µg/ml for crude extracts and 16-0.03 µg/ml for antibiotics. A number of wells were reserved in each plate for sterility control (no inoculum added), inoculum viability (no extract added) and the dimethyl sulfoxide inhibitory effect. Agar plate containing bacteria was incubated at 37°C for 24 h.

2.6 Statistical analysis

The results were expressed as mean±S.E.M of four determinations at each concentration for each sample. The IC_{50} values were calculated using the Microsoft Excel program. Statistical significance was calculated by one-way analysis of variance (ANOVA), followed by Dunnett's test.

3. Results and Discussion

Thirteen Thai crops were extracted with four solvents separately [(95% EtOH, 50% EtOH, water (W) and hot water (HW)] to obtain 112 extracts. The extracts were tested for anti-allergic effect using RBL-2H3 cells and anti-microbial activity. Among these extracts, the mango seed in 50% EtOH possessed the highest anti-allergic activity against antigen-induced β-hexosaminidase release as a marker of degranulation in RBL-2H3 cells with an IC_{50} value of 7.5± 0.8 µg/ml, followed by banana (W, IC_{50} = 13.5±2.4 µg/ml), okra (W, IC_{50} = 13.6±3.1 µg/ml), jampadah skin (HW, IC_{50} = 13.8±3.9 µg/ml), tamarind seed coat (HW, IC_{50} = 14.2±3.1 µg/ml) and jampadah flesh (W, IC_{50} = 14.6±3.1 µg/ml). These

Table 1. Anti-allergic activity of some Thai crops on antigen-induced degranulation in RBL-2H3 cells

Sample	Plant name	Part-used	Solvent	%Yield of extract (w/w)	Anti-allergy activity	
					% Inhibition at 100 µg/ml	IC_{50} ±SEM (µg/ml)
1	Banana (*Musa sapientum*)	peel	95% EtOH	0.97	30.45±3.88	>100
			50% EtOH	1.16	31.26±3.87	>100
			W	0.41	81.15±3.19	62.0±1.0
			HW	1.26	57.01±4.96	-*
		flesh	95% EtOH	1.14	55.65±10.48	-
			50% EtOH	1.17	40.59±4.52	>100
			W	0.26	28.72±11.68	>100
			HW	0.85	71.76±3.89	-
2	Ripe banana	peel	95% EtOH	0.61	60.84±4.73	-
			50% EtOH	2.20	56.54±5.39	-
			W	1.26	35.00±4.48	>100
			HW	1.38	53.82±6.64	-
		flesh	95% EtOH	6.87	78.14±2.38	-
			50% EtOH	4.13	58.80±3.67	-
			W	2.87	84.60±9.09	13.5±2.4
			HW	0.80	47.35±6.05	>100

Table 1. Continued

Sample	Plant name	Part-used	Solvent	%Yield of extract (w/w)	Anti-allergy activity	
					% Inhibition at 100 µg/ml	IC_{50} ±SEM (µg/ml)
3	Okra (*Hibiscus esculentus*)	pod	95% EtOH	1.69	53.92±4.61	-
			50% EtOH	1.24	90.79±6.74	43.9±2.6
			W	0.14	83.33±2.08	13.6±3.1
			HW	1.28	41.99±2.89	>100
4	Jackfruit (*Artocarpus heterophyllus*)	skin	95% EtOH	8.12	89.30±8.86	22.6±3.2
			50% EtOH	6.39	64.69±3.57	-
			W	3.47	86.91±2.72	22.7±1.6
			HW	3.62	67.32±4.09	-
		flesh	95% EtOH	10.84	61.15±6.88	-
			50% EtOH	9.43	98.76±5.07	34.1±2.5
			W	7.73	82.00±1.27	41.3±1.9
			HW	4.76	84.68±5.83	44.3±3.0
	Jackfruit	seed	95% EtOH	3.87	93.89±3.90	53.4±1.3
			50% EtOH	4.56	91.44±1.81	44.7±2.1
			W	2.97	73.49±3.62	-
			HW	3.42	47.59±0.88	>100
5	Germinated rice (*Oryza sativa*)	grain	95% EtOH	0.43	73.38±3.09	-
			50% EtOH	0.96	86.59±3.72	60.7±2.5
			W	0.11	65.70±2.37	-
			HW	3.14	22.02±6.87	>100
6	Rambutan (*Nepheleum lappaceum*)	peel	95% EtOH	9.48	77.57±1.49	-
			50% EtOH	10.41	47.59±0.88	>100
			W	3.98	39.56±6.50	>100
			HW	3.19	65.71±7.23	-
		flesh	95% EtOH	12.78	83.36±1.60	30.9±0.7
			50% EtOH	9.58	64.23±4.19	-
			W	8.82	83.26±1.60	53.6±1.3
			HW	11.13	87.43±4.42	62.1±1.2
		seed	95% EtOH	3.94	61.79±6.72	-
			50% EtOH	3.64	33.71±4.94	>100
			W	4.55	66.97±3.19	-
			HW	6.48	67.40±3.84	-
7	Fan palm fruit (*Borassus flabellifer*)	flesh (endosperm)	95% EtOH	6.83	58.58±1.59	-
			50% EtOH	5.93	56.27±2.71	-
			W	5.31	85.96±3.41	31.6±2.3
			HW	1.85	46.83±5.12	>100
8	Jampadah (*Artocarpus integer*)	skin	95% EtOH	6.02	77.63±4.00	-
			50% EtOH	6.90	100.76±3.38	42.9±1.8
			W	1.70	81.75±3.12	37.0±1.7
			HW	2.06	93.47±9.66	13.8±3.9
	Jampadah	flesh	95% EtOH	10.29	77.23±3.63	-
			50% EtOH	6.92	67.02±2.71	-
			W	4.38	80.40±2.37	14.6±3.1
			HW	7.78	69.00±2.39	-

Table 1. Continued

Sample	Plant name	Part-used	Solvent	%Yield of extract (w/w)	Anti-allergy activity	
					% Inhibition at 100 µg/ml	IC_{50} ±SEM (µg/ml)
		seed	95% EtOH	4.95	82.55±0.83	58.1±0.8
			50% EtOH	3.36	98.63±5.66	49.5±2.1
			W	2.03	79.49±2.69	60.9±1.0
			HW	1.80	79.13±3.40	-
9	Durian (*Durio zibethinus*)	skin	95% EtOH	3.19	60.86±1.12	-
			50% EtOH	4.01	77.59±7.17	-
			W	0.23	63.82±2.42	-
			HW	1.02	52.57±4.41	-
10	Huasa potato (*Coleus parvifolius*)	tuber	95% EtOH	1.28	58.19±6.97	-
			50% EtOH	0.93	59.70±3.01	-
			W	0.63	75.34±4.56	-
			HW	0.95	64.86±3.57	-
11	Tamarind (*Tamarindus indica*)	flesh	95% EtOH	7.06	86.58±1.35	37.8±1.6
			50% EtOH	8.94	38.09±8.69	>100
			W	4.48	81.44±2.50	51.6±2.5
			HW	12.45	91.77±1.95	60.6±0.8
12	Tamarind seed	seed coat	95% EtOH	19.62	76.57±1.72	-
			50% EtOH	20.11	81.25±2.04	44.1±1.5
			W	9.06	99.16±1.30	46.1±1.6
			HW	10.48	98.67±4.66	14.2±3.1
		kernel	95% EtOH	2.67	27.28±5.37	>100
			50% EtOH	5.12	51.19±1.47	-
			W	7.23	68.39±5.02	-
			HW	7.09	60.38±1.86	-
13	Coconut (*Cocos nucifera*)	flesh	95% EtOH	3.41	54.83±3.10	-
			50% EtOH	2.68	56.35±4.19	-
			W	3.07	60.87±3.43	-
			HW	2.66	46.49±2.81	>100
14	Mango (*Mangifera indica*)	peel	95% EtOH	2.68	76.42±5.11	-
			50% EtOH	4.56	78.31±5.03	-
			W	1.66	73.30±4.09	-
			HW	2.54	63.53±2.29	-
		flesh	95% EtOH	5.17	85.11±1.83	60.7±0.6
			50% EtOH	1.59	83.57±5.99	55.5±2.2
			W	2.20	82.09±5.23	51.2±2.6
			HW	5.04	66.41±9.51	-
		seed	95% EtOH	9.20	97.10±0.96	21.5±1.8
			50% EtOH	6.72	85.07±3.63	7.5±0.8
			W	4.49	89.01±0.97	40.4±1.4
			HW	2.56	78.85±2.19	-
15	Fan palm fruit (*Borassus flabellifer*)	skin	95% EtOH	7.48	75.99±4.94	-
			50% EtOH	7.57	62.11±5.64	-
			W	4.54	74.96±3.79	-
			HW	5.82	77.47±2.04	>100

Table 1. Continued

Sample	Plant name	Part-used	Solvent	%Yield of extract (w/w)	Anti-allergy activity	
					% Inhibition at 100 µg/ml	IC_{50} ±SEM (µg/ml)
		flesh	95% EtOH	3.60	51.71±2.68	-
			50% EtOH	2.10	57.48±3.28	-
			W	0.74	59.69±2.14	-
			HW	0.80	28.09±0.85	-
16	Dioscorea (*Dioscorea membranacea*)	tuber	95% EtOH	-	82.01±3.86	37.5±2.6
			50% EtOH	1.68	52.73±4.11	-
			W	3.78	85.25±0.97	33.9±0.6
			HW	-	59.07±1.92	-
	Ketotifen fumarate	-	-		68.20±1.50	20.2±1.3

* (-) = not determined

Table 2. MIC values (µg/ml) of some Thai crops against selected microorganisms using agar dilution assay

Sample	Solvent	MIC (µg/ml)		
		S. aureus	*B. subtilis*	*P. aeruginosa*
Rambutan peel	95% EtOH	500	-*	-
	50% EtOH	500	-	-
	Water	500	500	-
	Hot water	250	500	-
Rambutan flesh	95% EtOH	-	.>2000	-
Banana peel	Hot water	500	-	-
Mango peel	95% EtOH	-	1000	-
	50% EtOH	2000	2000	-
Mango seed	95% EtOH	250	500	1000
	50% EtOH	250	500	2000
	Water	250	500	-
	Hot water	250	500	-
Tamarind kernel	95% EtOH	-	>2000	-
Tamarind seed-coat	95% EtOH	500	1000	-
	50% EtOH	500	500	-
	Water	500	1000	-
	Hot water	500	1000	-
Tetracycline	-	1	2	-
Norfloxacin	-	-	-	0.5

* (-) = not determined

crops possessed anti-allergic effects higher than ketotifen fumarate, a positive control (IC_{50} = 20.2±1.3 µg/ml). Other plants possessed IC_{50} values from 21.5->100 µg/ml (Table 1). The results also revealed the importance of the solvent used for the extraction of each plant. The 50% EtOH is an appropriate solvent for the extraction of mango seed, whereas water is suitable for banana fruit and jampadah flesh; and hot water is the best for tamarind seed coat and jampadah skin. Moreover, the plants showing high anti-allergic effects also possessed marked anti-bacterial activity.

Rambutan peel, mango peel, mango seed and tamarind seed coat exhibited appropriate anti-bacterial activity against *S. aureus*, *B. subtilis* and *P. aeruginosa* with MIC values ranging from 250-2,000 µg/ml (Table 2), but did not show any effect towards *E. coli* and *C. albicans* (data not shown). Mango seed and tamarind seed have been used in Thai traditional medicine for wound healing, whereas banana and okra have been used as tonic for the treatment of peptic ulcer (Pengcharoen, 2002). Some stilbenes isolated from the aerial parts of *A. integ*er (jampadah) have been reported to have anti-malarial activity against *Plasmodium falciparum* (Choosak *et al.*, 2000). Stilbene derivatives (piceatannol, resveratrol) isolated from Korean rhubarb (*Rheum undulatum*) showed anti-allergic effect against antigen-induced mast cell degranulation (Matsuda *et al.*, 2004). Therefore, stilbene compounds contained in jampadah skin and flesh might be responsible for the anti-allergic activity of this plant since this kind of compounds could be easily dissolved in water. The components that are responsible for the anti-allergic and anti-microbial activities of these promising plants will be further investigated.

This study indicates that some Thai crops may have potential as functional foods and neutraceuticals for the treatment of allergy, allergy-related diseases and some bacterial infections, especially Gram-positive bacteria.

Acknowledgements

The authors are grateful to the Reverse Brain Drain program, National Science and Technology Development Agency, Thailand for financial support. We also thank the Faculty of Pharmaceutical Sciences and Faculty of Agro-Industry, Prince of Songkla University, for providing laboratory facilities.

References

Boonlaksiri, C., Oonanant, W., Kongsaeree. P., Kittakoop, P., Tanticharoen, M., and Thebtaranonth, Y. 2000. An anti-malarial stilbene from *Artocarpus integer*. Phytochemistry, 54, 415-417.

Cheong, H., Choi, E.J., Yoo, G.S., Kim, K.M., and Ryu, S.Y. 1998. Desacetylmatricarin, an anti-allergic compound from *Taraxacum platycarpum*. Planta Medica, 64, 577-578.

Goldsby, RA., Kindt, T.J., Osborne, B.A., and Kuby, J. 2002. Immunology. fifth edition. W.H. Freeman and Company, New York, pp. 361-370.

Lorian, V. 1996. Antibiotics in Laboratory Medicines, fourth ed. Williams and Wilkins, Baltimore.

Matsuda, H., Tewtrakul, S., Morikawa, T., Nakamura, A., and Yoshikawa, M. 2004. Anti-allergic principles from Thai zedoary: structural requirements of curcuminoids for inhibition of degranulation and effect on the release of TNF-α and IL-4 in RBL-2H3 cells. Bioorganic & Medicinal Chemistry, 12, 5891-5898.

Matsuda H., Tewtrakul S., Morikawa T., and Yoshikawa, M. 2004. Anti-allergic activity of stilbenes from Korean rhubarb (*Rheum undulatum* L.): structure requirements for inhibition of antigen-induced degranulation and their effects on the release of TNF-a and IL-4 in RBL-2H3 cells. Bioorganic & Medicinal Chemistry, 12, 4871-4876.

Pengcharoen, O. 2002. Technology Chao Barn. Matichon Press, Bangkok, pp. 42-43.

Tada, T., and Okumura, K. 1971. Regulation of homocytotropic antibody formulation in the rat. I. Feed-back regulation by passive administered antibody. The Journal of Immunology, 106, 1002-1011.