รายงานวิจัยฉบับสมบูรณ์

โครงการ
“ฤทธิ์ต้านการอักเสบและต้านภูมิแพ้ของผลไม้ไทย”

Anti-inflammatory and anti-allergic effects of some selected Thai fruits

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Contents

บทคัดย่อ

การศึกษาฤทธิ์ต้านการอักเสบและต้านภูมิแพ้ของผลไม้ไทย 9 ชนิด ได้แก่ ส้มโอ ส้มโชกุน ชมพู่พลาสติก ละมุด ฝรั่ง มะละกอ เงาะ มังคุด ลองกอง โดยฤทธิ์ต้านการอักเสบจะใช้วิธีตรวจวัดปริมาณ nitric oxide และ prostaglandin E_2 ที่ macrophage (RAW 264.7) cell สร้างขึ้น ฤทธิ์ต้านภูมิแพ้ ศึกษาโดยวัดการยับยั้งการเกิด degranulation ของ mast cell ซึ่งจะหลั่งสาร β-hexosaminidase ที่เป็นตัวชี้วัดการเกิดการแพ้ และการยับยั้งการหลั่ง TNF-α จาก rat basophil leukemia (RBL)-2H3 cells ผลการทดลองพบว่าสารสกัดชั้นแอลกอฮอล์ของฝรั่งมีฤทธิ์ยับยั้ง nitric oxide มากที่สุด สารสกัดชั้นน้ำของฝรั่ง สารสกัดชั้นแอลกอฮอล์ของส้มโชกุนและมะละกอ มีฤทธิ์รองลงมาตามลำดับ ส่วนผลไม้อื่นมีฤทธิ์น้อย การยับยั้ง PGE_2 พบว่าผลไม้ทั้ง 9 ชนิดมีฤทธิ์น้อย (IC_{50}>100 µg/ml) แสดงว่า ฝรั่ง ส้มโชกุน และมะละกอ มีฤทธิ์ต้านการอักเสบโดยยับยั้งการหลั่ง nitric oxide ส่วนผลต้านภูมิแพ้ พบว่า สารสกัดชั้นน้ำของมังคุดมีฤทธิ์ดีที่สุดในการยับยั้งการหลั่งสาร β-hexosaminidase ตามด้วยสารสกัดชั้นแอลกอฮอล์ของมังคุดสารสกัดชั้นน้ำของเงาะ สารสกัดชั้นแอลกอฮอล์ของชมพู่พลาสติกและเงาะตามลำดับ ส่วนสารสกัดผลไม้อื่นมีฤทธิ์ปานกลางถึงน้อย ฤทธิ์ยับยั้งการหลั่ง TNF- α พบว่าสารสกัดผลไม้ทั้งหมดมีฤทธิ์น้อย แสดงว่ามังคุด เงาะ และชมพู่พลาสติกมีฤทธิ์ต้านภูมิแพ้โดยยับยั้ง degranulation ของ mast cell ซึ่งเป็นระยะต้นของภูมิแพ้

Abstract

Eighteen extracts of aqueous and ethanolic (EtOH) extracts of nine fruits including *Nephelium lappaceum* (Rambutan), *Syzygium aquem* (Water rose apple), *Garcinia mangostana* (Mangosteen), *Aglaia dookkoo* (Long-kong), *Guava sativa* (Guava), *Carica papaya* (Papaya), *Achras sapota* (Sapodilla plum), *Citrus reticulata* (Tangerine) and *Citrus maxima* (Pomelo) were tested for their anti-inflammatory activity against NO and PGE_2 release using RAW264.7 macrophage cells. Anti-allergic activities assay were evaluated for β–hexosaminidase release as a marker of degranulation and the inhibitory effect on TNF-α production in rat basophil leukemia (RBL)-2H3 cells.

The results indicated that The ethanolic extract of Guava possessed appreciable anti-NO activity, followed by the water extract of guava, ethanolic extracts of tangerine and papaya respectively, whereas other plant extracts exhibited low activity. For PGE_2 inhibitory effect, it was found that all of nine fruits showed mild effect (IC_{50} > 100 μg/ml). It is suggested that some fruits such as guava, tangerine and papaya have potential for treatment of inflammation by inhibition on NO production.

For anti-allergic effects, the result showed that the water extract of mangosteen possessed appreciable effect on β-hexosaminidase release activity, followed by ethanolic extract of mangosteen, water extract of rambutan, ethanolic extract of water rose apple and rambutan respectively,whereas other fruit extracts exhibited moderate to mild activity.

For the inhibitory effect on TNF-α production, it was showed that all fruit extracts exhibited mild activity. This finding suggests that mangosteen, rambutan and water rose apple have antiallergic activity by inhibiting mast cell degranulation in the early phase of allergic reaction.

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Executive summary

Introduction

The inflammatory response consists of vascular and cellular reactions. These reactions are mediated by chemical factors derived from plasma proteins or cells (including mast cells platelets, neutrophils and monocyte/macrophage). They are triggered by bacteria products or host protein. Chemical mediators include vasoactive amines (histamine, serotonin), arachidonic acid derivatives (protaglandins, leukotrienes) and cytokines (tumor necrosis factor and interleukin-1). Inflammatory mediators such as nitric oxide (NO) is produced in large quantities during host defense and immunologic reactions. It has cytotoxic properties and is generated by activated macrophages.

Prostaglandin E_2 (PGE_2) is an extensively studied prostaglandin owing to its predominance in inflammation, autoimmune disease, and sepsis. PGE_2 is believed to play crucial roles, since chemical mediators invoke PGE_2 synthesis in monocytes and neutrophils at inflammation sites.

Macrophages possess certain types of receptors that recognize differential carbohydrate patterns on foreign cells. They also have receptors for specific bacterial products such as lipopolysaccharide (LPS). Macrophages can kill pathogens directly by phargocytosis and indirect via the secretion of various pro-inflammatory mediators such as nitrogen species and proinflammatory cytokines (TNF-α).

Allergy is an immunological reaction to a foreign antigen (allergen) that causes tissue inflammation and organ dysfunction. The early-phase mediators of allergy are histamine and serotonin, and the late phase mediators are lymphokine and monokines. Upon the degranulation of mast cells, β-hexosaminidase is also released along with histamine; therefore, this enzyme is used as a marker for mast cell degranulation.

Fruits are rich with vitamins C, A, B and E and polyphenols. Most of polyphenol are flavonoids mainly in ester and glycoside forms.

The purpose of this study is to investigate the effects of some selected Thai fruits on anti-inflammatory for NO inhibitory effect and LPS-induced PGE_2 release using RAW264.7 macrophage cells. Anti-allergic activities assay were evaluated for β-hexosaminidase release as a marker of degranulation and the inhibitory effect on TNF-α production in rat basophil leukemia (RBL)-2H3 cells.

Materials and Methods

The water and ethanolic extracts of nine fruits were prepared for testing. RAW.264.7 cells were treated with various test samples and stimulate with LPS. Supernatants were collected and assayed for nitric oxide release by Greiss reaction and PGE_2 production by commercial ELISA kit.

The anti-allergic effects on LPS stimulated of mast cell degranulation and cytokine release were evaluated. RBL-2H3 cells were treated with various test samples and stimulate with antigen. The cell supernatants were used to determine mast cell degranulation using β-hexosaminidase release assay and the concentrations of TNF-α using commercial ELISA kit.

Cell viability was evaluated by an MTT assay.

Results

The ethanolic extract of guava possessed appreciable anti-NO activity, followed by the water extract of guava, ethanolic extract of tangerine and papaya, respectively; whereas other plant extracts exhibited low activity. For PGE_2 inhibitory effect, it was found that all of nine fruits showed mild effect (IC_{50} > 100 μg/ml). It is suggested that some fruits such as guava, tangerine and papaya have potential for treatment of inflammation by inhibition on NO production.

For anti-allergic effects, the result showed that the water extract of mangosteen possessed appreciable effect on β-hexosaminidase release activity, followed by ethanolic extract of mangosteen, water extract of rambutan, ethanolic extract of water rose apple and rambutan respectively. Other fruit extracts exhibited moderate to mild activity.

For the inhibitory effect on TNF-α production, it was showed that all fruit extracts exhibited mild activity. This finding suggests that mangosteen, rambutan and water rose apple have antiallergic activity against mast cell degranulation in the early phase of allergy.

Research work

Anti-inflammatory and anti-allergic effects of some selected Thai fruits

1. Introduction

The inflammatory process is best viewed as the body's response to cellular injury, and the function of inflammation is tissue healing. It can occur anywhere, acutely in the skin around a wound or in less visible sites such as the lining of the middle ear, chronically it can be related to persistent infection, ulceration, mechanical or chemical irritation (http://www.answer.com/topic/inflammation). The inflammatory response consists of a vascular and a cellular reaction. These reactions are mediated by chemical factors derived from plasma proteins or cells (including mast cells platelets, neutrophils and monocyte/macrophage). They are triggered by bacteria products or host protein. Chemical mediators include vasoactive amines (histamine, serotonin), arachidonic acids (protaglandins, leukotrienes) and cytokines (tumor necrosis factor and interleukin-1). Inflammatory is a local reactive change that involves the release of antibacterial agents from nearby cells that defend the host against infection (http://www.adha.org).

Inflammatory mediators, such as nitric oxide (NO) is produced in large quantities during host defense and immunologic reactions. NO shows cytotoxic properties and is generated by activated macrophages, it is likely to have a role in non-specific immunity. Nitric oxide posseses numerous biological properties in many cell types ranging from bactericidal effects of macrophages, signal transduction during inflammation and cytoprotection (Clancy and Amin, 1998).

In inflammation processes, prostaglandin E_2 (PGE_2) is believed to play crucial roles, since chemical mediators invoke PGE_2 synthesis in monocytes at inflammation sites (Niclols *et al*., 1988). PGE_2 is an extensively studied prostaglandin owing to its predominance in inflammation, autoimmune disease, and sepsis.

The process of acute inflammation is initiated by cells already present in all tissues such as macrophage and dendritic cells. Macrophages posses certain types of receptors, that

recognize differential carbohydrate patterns on foreign cells. They also have receptors for specific bacterial products such as lipopolysaccharide (LPS). Macrophages can kill pathogens directly by phargocytosis and indirect via the secretion of various pro-inflammatory mediators such as nitrogen species and proinflammatory cytokines (TNF-α) (Fujiwara and Kobayashi, 2005).

Allergy is an immunological reaction to a foreign antigen (allergen) that causes tissue inflammation and organ dysfunction. The early-phase mediators of allergy are histamine and serotonin, and the late phase mediators are lymphokines and monokines.

Rat basophil leukemia (RBL-2H3) cells display properties of mucosal-type mast cells. The RBL-2H3 cells contain several hundred thousand IgE receptors on the membrane surface, and after sensitization with allergen, the cells respond to antigen and release chemical immune mediators such as histamine, and tumor necrosis factor (TNF-α). Upon the degranulation of mast cells, β-hexosaminidase is also released along with histamine; therefore, this enzyme is used as a marker for mast cell degranulation (Matsuda *et al*., 2002).

Fruits are abundant in nutrients, such as fiber, potassium, folate, and vitamin C. Moreover, they also contain carotenoids and polyphenols, which act as antioxidants within the body. (Szekancecz and Koch 2007**,** Jeong et al 2010). We can prevent heart disease and cancer by eating more fruits and vegetables because fruits and vegetables show anti-inflammatory effect because both heart disease and cancer are pro-inflammatory diseases (Hu, 2003**,** Van and Pivonka, 2000).

In this study, nine fruits were selected for anti-inflammatory testing on nitric oxide (NO) inhibitory effect and LPS-induced PGE_2 release using RAW264.7 cell line. Moreover anti-allergic effects against β–hexosaminidase release as a marker of mast cell degranulation and the inhibitory effect on TNF-α production in rat basophil leukemia (RBL)-2H3 cells were also investigated.

Thai fruits in this study and their bioactive components are as follow; (Charoensiri *et al*., 2009)

***Nephelium lappaceum* Linn. (Rambutan)**

Rambutan fruit contains carbohydrate, protein, fat, phosphorus, iron, calcium and fiber. Rambutan also contains polyphenol, catechin, phytate and vitamin C which known as antioxidants.

***Syzygium aqueum* Burm. f (Water rose apple)**

The water rose apple is mainly consumed by children, the appeal being largely its thirst-relieving character. In Indonesia, the fruits are sold in markets in piles or skewered on slender bamboo sticks. Superior types are sometimes served sliced in salads. No nutritional information currently available.

***Garcinia mangostana* Linn. (Mangosteen)**

Mangosteen is good in vitamin C. Vitamin C is a powerful water soluble anti-oxidant. Consumption of fruits rich in vitamin C helps body develop resistance against flu-like infectious agents and scavenge harmful, pro-inflammatory free radicals. Fresh fruit is a very good source of B-complex vitamins such as thiamin, niacin and folates. These vitamins are acting as cofactors help body metabolize carbohydrates, proteins and fats. Mangosteen also contains a very good amount of minerals like potassium, manganese and magnesium. Potassium in an important component of cell and body fluids helps control heart rate and blood pressure; thus offers protection against stroke and coronary heart diseases.

***Aglaia dookkoo* Griff. (Long-kong)**

The fruit contains a variety of nutrients, including proteins and carbohydrates, a low fat content and a high percentage of vitamins and minerals (Sabah, 2004).

***Guava sativa* Linn. (Guava)**

It enriches the diet of millions of people as a good source of lycopene, beta-carotene, vitamin C and soluble fibre (Satiawan *et al*., 2001). Guava also contains ellagic acid, gallic acid conjugates, and quercetin glycosides.

***Carica papaya* Linn. (Papaya)**

Papaya is a rich source of antioxidant nutrients like carotenes, vitamin C, vitamin E, minerals, fiber, etc. All these nutrients boost the health of the cardiovascular system, and providing protection against colon cancer.

***Achras sapota* Linn. (Sapodilla plum)**

Sapodilla is high in fiber, iron, and calcium. Two unusual polyphenolic compounds with high antioxidant activity, methyl 4-O-galloylchlorogenate and 4-O-galloylchlorogenic acid (Talcott *et al*., 2003), have been identified in sapodilla. 5-caffeoylquinte (CQA) was also found in small quantities in the sapodilla (Pontes *et al*., 2002). In addition, sapodilla contains catechin conjugates and polyphenols (Mahattanatawee *et al*., 2006).

***Citrus reticulata* Blanco (Tangerine)**

This fruit is a good source of thiamin and a very good source of vitamin A. The fruit contains ascorbic acid, enzymes, bioflavonoids, rich in minerals like chromium, potassium, and magnesium. (Janick and Paull, 2008)

***Citrus maxima* Merr. (Pomelo)**

The fruit contain mostly vitamin E, bioflavonoids and carotenoids (http://www.prlog.org/10753283).

2. Materials and methods

2.1 Plant materials

Water rose apple was bought from Namom district, Songkhla in June 2009. Rambutan, mangosteen and long-kong were bought from Hatyai district, Songkhla in August. Pomelo was bought from Hatyai district in September. Guava, papaya and tangerine were bought from Hatyai district in October, whereas sapodilla plum was bought from Lansaka district, Nakornsrithammarach in November. Mature fruits were washed with clean water and chopped into small pieces. After that, they were extracted using the juice extractor to obtain the fruit juice. In situation where the filtrate appeared to be very cloudy, the filtrate was centrifuged to obtain a clear supernatant liquid. The fruit juices were then freeze dried, whereas the pomaces

were macerated further with ethanol 3 times to obtain the ethanolic extract of each fruit. The filtrates of the fruits were evaporated under reduce pressure and freeze dry. The extracts were stored at −20 °C.

2.2 Reagents

2.2.1 Antiinflammatory activity assay

Lipopolysaccharide (LPS, from *Escherichia coli*), RPMI-1640 medium, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT), indomethacin (non steroidal anti-inflammatory drug, NSAID) and phosphate buffer saline (PBS) were purchased from Sigma Aldrich (Sigma Aldrich, Missouri, USA). Fetal calf serum (FCS) was bought from Gibco (Invitrogen, California,USA). Penicillin-streptomycin was purchased from Invitrogen (Invitrogen, California, USA). 96-Well microplates were obtained from Nunc (Nunc, Birkrød, Denmark). ELISA test kits of PGE_2 were from R&D systems (R&D systems, Minnesota, USA). Other chemicals were from Sigma Aldrich (Sigma–Aldrich, Missouri, USA).

2.2.2 Anti-allergic activity assay

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.

2.3 Assay for NO inhibitory effect from RAW264.7 cells (Banskota *et al*., 2003)

Inhibitory effect on NO production by murine macrophage-like RAW264.7 cells was evaluated using a modified method from that previously reported. Briefly, the RAW264.7 cell line (purchased from Cell Lines Services) was cultured in RPMI medium supplemented with 0.1% sodium bicarbonate and 2 mM glutamine, penicillin G (100 units/ml), streptomycin (100 μg/ml) and 10% FCS. The cells were harvested with trypsin-EDTA and diluted to a suspension in a fresh medium. The cells were seeded in 96-well plates with $1x10^5$ cells/well and allowed to adhere for 1 h at 37 ºC in a humidified atmosphere containing 5% CO_2. After that, the medium was replaced with a fresh medium containing 100 μg/ml of LPS together with the test samples at various concentrations (10–100 μg/ml for crude extracts) and was then

incubated for 48 h. NO production was determined by measuring the accumulation of nitrite in the culture supernatant using the Griess reagent. The pink color of an azo product after adding the Griess reagent was measured at wavelength 570 nm using the microplate reader. Cytotoxicity was determined using the MTT colorimetric method. Briefly, after 48 h incubation with the test samples, MTT solution (10 µl, 5 mg/ml in PBS) was added to the wells. After 4 h incubation, the medium was removed, and isopropanol containing 0.04 M HCl was then added to dissolve the formazan production in the cells. The optical density of the formazan solution was measured with a microplate reader at 570 nm. The test compounds were considered to be cytotoxic when the optical density of the sample-treated group was less than 80% of that in the control (vehicle-treated) group. Indomethacin at concentrations of 1-100 µM (non-steroidal anti-inflammatory drug, NSAID) was used as a positive control. The stock solution of each test sample was dissolved in DMSO, and the solution was added to the medium RPMI (final DMSO is 1%). % Inhibition was calculated using the following equation and IC_{50} values were determined graphically (n = 4):

$$\text{Inhibition (\%)} = \frac{A - B}{A - C} \times 100$$

A-*C*: NO_2^- concentration (µM) [*A* : LPS (+), sample (-); *B* : LPS (+), sample(+); *C* : LPS (-), sample (-)].

2.4 Inhibitory effects on LPS-induced PGE_2 release from RAW264.7 cells

Briefly, the RAW264.7 cell line was cultured in RPMI medium supplemented with 0.1% sodium bicarbonate and 2 mM glutamine, penicillin G (100 units/ml), streptomycin (100 µg/ml) and 10% FCS. The cells were harvested with trypsin-EDTA and diluted to a suspension in a fresh medium. The cells were seeded in 96-well plates with 1.0 $x10^5$ cells/well and allowed to adhere for 1 h at 37 ºC in a humidified atmosphere containing 5% CO_2. After that, the medium was replaced with a fresh medium containing 100 µg/ml of LPS together with the test samples at various concentrations (10–100 µg/ml), and was then incubated for 48 h. The test samples were dissolved in RPMI medium. The supernatant was transferred into 96-well ELISA plate and then PGE_2 concentrations were determined using commercial ELISA kits. The test sample was dissolved in DMSO, and the solution was added to RPMI. The inhibition on PGE_2 releases was calculated and IC_{50} values were determined graphically.

2.5 Inhibitory effects of plant extracts on the release of β-hexosaminidase from RBL-2H3 cells (Matsuda *et al*., 2002)

Inhibitory effects on the release of β-hexosaminidase from RBL-2H3 cells (purchased from ATCC) were evaluated by the following modified method. 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. After that, 20 μl of test sample solution was added to each well and incubated for 10 min, followed by 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 was used as a positive control. The inhibition (%) of the release of β-hexosaminidase by the test samples was calculated by the following equation, and 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 (–)

2.6 Inhibitory effects on antigen-induced TNF-α release from RBL-2H3 cells

Inhibitory effects on the release of TNF-α from RBL-2H3 (the late phase allergic reaction) were evaluated by the method reported previously. RBL-2H3 cells (2 x 10^5 cells/well) were sensitized with anti-DNP-IgE as described above. The cells were washed with MEM containing 10% FCS, penicillin (100 units/mL) and streptomycin (100 μg/mL), and exchanged with 320 μL of fresh medium. Then 40 μL of test sample solution, and 40 μL of antigen (DNP-BSA, final concentration was 10 μg/mL) were added to each well and incubated at 37°C for 4 h. The supernatant was transferred into 96 well ELISA plate and then TNF-α concentrations were determined using commercial ELISA kits. The test samples were dissolved in DMSO, and the solution was added to MEM (final DMSO was 0.1%). The inhibition on TNF-α production was calculated by the following equation, and IC_{50} values were determined graphically:

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

Control (C): DNP-BSA (+), Test sample (–); Test (T): DNP-BSA (+), Test sample (+);

Normal (N): DNP-BSA (–), Test sample (–)

2.7. 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

The percent yields of each fruit extract are shown in Table 1. Eighteen extracts of aqueous and ethanolic (EtOH) extracts of nine fruits including *Nephelium lappaceum* (Rambutan), *Syzygium aquem* (Water rose apple), *Garcinia mangostana* (Mangosteen), *Aglaia dookkoo* (Long-kong), *Guava sativa* (Guava), *Carica papaya* (Papaya), *Achras sapota* (Sapodilla plum), *Citrus reticulata* (Tangerine) and *Citrus maxima* (Pomelo) were tested for

their anti-inflammatory activity against NO and PGE_2 release using RAW264.7 macrophage cells. The results indicated that The EtOH extract of Guava possessed appreciable anti-NO activity with an IC_{50} value of 23.5 µg/ml, followed by the water extract of Guava (IC_{50} = 30.1 µg/ml), EtOH extract of Tangerine (IC_{50} = 31.3 µg/ml) and EtOH extract of Papaya (IC_{50} = 34.2 µg/ml), respectively, whereas other plant extracts exhibited IC_{50} values ranging from 45.4->100 µg/ml. For the positive control, indomethacin (an NSAID) had an IC_{50} of 25 µM (8.9 µg/ml) (Table 2).

For PGE_2 inhibitory effect, it was found that all of nine fruits showed mild effect (IC_{50} > 100 µg/ml) on inhibition of PGE_2 production (Table 3).

For anti-allergic effects of some selected Thai fruits, the result showed that the water extract of *Garcinia mangostana* (Mangosteen) possessed appreciable effect on β-hexosaminidase release activity with an IC_{50} value of 13.5 µg/ml, followed by *Garcinia mangostana* (EtOH, IC_{50} = 17.4 µg/ml), *Nephelium lappaceum* (Rambutan) (water, IC_{50} = 17.7 µg/ml), *Syzygium aqueum* (Water rose apple, EtOH, IC_{50} = 21.8 µg/ml) and *Nephelium lappaceum* (EtOH, IC_{50} = 36.4 µg/ml), respectively. Whereas other fruit extracts exhibited moderate to mild activity (IC_{50} from 51.9->100 µg/ml). The clinical used drug, ketotifen fumarate possessed an IC_{50} value of 20.2 µg/ml. For the inhibitory effect on TNF-α production, it was showed that all fruit extracts exhibited mild activity with IC_{50} value ranging from 93.8 -> 100 µg/ml.

. It is suggested that some fruits such as guava, tangerine and papaya have potential for treatment of inflammation by inhibition on NO production. It has been reported that guava contains some polyphenolic compounds such as tannin and catechin which may responsible for anti-inflammatory effect of this plant. Polyphenols such as flavonoids have also been reported for anti-inflammatory and anti-oxidant activities (Urquiaga and Leighton, 2000). Papaya has been reported to contain β-carotene, vitamin A and vitamin C which are anti-oxidant agents (Isabell et al., 2010). Tangerine has been found to contain vitamins A and C (http://prlog.org/10749187). Therefore, these kinds of compounds might be responsible for anti-inflammatory activity of these fruits.

For anti-allergic effects, it was found that mangosteen, rambutan and water rose apple inhibited mast cell degranulation in the early phase of allergic reaction. Mangosteen contains several bioactive compounds such as vitamin C, quercetin, luteolin etc. Rambutan contains vitamin C, phytate and polyphenol. Although water rose apple has no nutrition information, but there are high polyphenols in rose apple. Various compounds may be involved in different mechanisms to suppress the allergic response. Polyphenols, a rich substance of bioflavonoids, is present in fruits and vegetables and has anti-allergy properties of the kind that actually prevent allergic reactions rather than treating the symptoms. Large doses of quercetin, 4 to 6 grams per day, may also be helpful to some allergy patients.

It has been reported that thirteen Thai crops extracted with four solvents separately [(95% EtOH, 50% EtOH, water (W) and hot water (HW)] possessed anti-allergic effect. Among these extracts, the mango seed in 50% EtOH showed 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 μg/ml, followed by banana (water, IC_{50} = 13.5 μg/ml), okra (water, IC_{50} = 13.6 μg/ml), jampadah skin (hot water, IC_{50} = 13.8 μg/ml), tamarind seed coat (hot water, IC_{50} = 14.2 μg/ml) and jampadah flesh (water, IC_{50} = 14.6 μg/ml), respectively (Tewtrakul *et al*., 2008).

In conclusion, tropical fruits are high in vitamins, minerals, fiber and phytochemicals. Guava, tangerine and papaya have anti-inflammatory activity via suppress the nitric oxide release in RAW264.7 cells. Mangosteen, rambutan and water rose apple inhibit β-hexosaminidase release in RBL-2H3 cells resulting in antiallergic activity.

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Table 1. Percent yield of fruit juice extracts and alcoholic extracts

Fruit	Weight after remove peel and stones (kg)	part	Pomaces Weight (kg)	Dry weight (g)	Yield (%w/w)
Water rose apple 1 kg	0.8	juice		8.17	1.02
		alcohol	0.45	6.08	1.35
Rambutan 6 kg	2.28	juice		172.56	7.57
		alcohol	0.56	22.71	4.05
Mangosteen 4.5 kg	2.0	juice		111.20	5.56
		alcohol	1.21	81.85	6.76
Long-kong 2 kg	1.52	juice		90.65	5.96
		alcohol	0.70	42.21	5.99
Pomelo 1.94 kg	1.1	juice		53.34	4.85
		alcohol	0.51	34.47	6.69
Guava 1 kg	0.9	juice		11.74	1.30
		alcohol	0.40	8.28	2.07
Papaya 2.2 kg	1.56	juice		8.74	0.56
		alcohol	0.88	20.74	2.36
Tangerine 2.7 kg	2.3	juice		78.76	3.42
		alcohol	1.10	25.77	2.34
Sapodilla plum 1.5 kg	1.2	juice		51.90	4.33
		alcohol	0.60	8.79	1.47

Table 2. Inhibitory activity on NO production[a] of Thai fruits

Sample	% Inhibition at various concentrations (μg/ml)						IC_{50} (μg/ml)
	Solvent	0	3	10	30	100	
(**1**) *Nephelium lappaceum*	Water	0.0 ± 2.0	-	12.8 ± 1.9	17.8 ± 2.0*	39.5 ± 3.8**	>100
(**2**) *Nephelium lappaceum*	EtOH	0.0 ± 2.0	-	11.3 ± 3.5	17.5 ± 3.2	46.0 ± 4.1**	>100
(**3**) *Syzygium aquem*	Water	0.0 ± 2.0	-	12.8 ± 1.5	17.5 ± 1.4*	40.8 ± 3.4**	>100
(**4**) *Syzygium aquem*	EtOH	0.0 ± 2.0	-	13.0 ± 1.5	18.8 ± 2.0*	38.8 ± 3.3**	>100
(**5**) *Garcinia mangostana*	Water	0.0 ± 2.0	-	12.8 ± 2.1	22.8 ± 2.6**	43.0 ± 2.5**	>100
(**6**) *Garcinia mangostana*	EtOH	0.0 ± 2.0	-	19.8 ± 1.5	22.3 ± 1.1**	43.3 ± 2.1**	>100
(**7**) *Aglaia dookkoo*	Water	0.0 ± 2.0	-	11.0 ± 1.0	14.5 ± 2.5*	46.5 ± 1.6**	>100
(**8**) *Aglaia dookkoo*	EtOH	0.0 ± 2.0	-	12.0 ± 2.5	19.5 ± 2.2**	42.0 ± 3.1**	>100
(**9**) *Guava sativa*	Water	0.0 ± 9.1	-	36.2 ± 2.6*	47.2 ± 2.0**	67.9 ± 1.6**	30.1
(**10**) *Guava sativa*	EtOH	0.0 ± 9.1	-	33.9 ± 6.6	56.9 ± 1.8**	73.4 ± 2.3**	23.5
(**11**) *Carica papaya*	Water	0.0 ± 9.1	-	25.7 ± 3.8	39.4 ± 2.6**	64.2 ± 3.6**	46.8
(**12**) *Carica papaya*	EtOH	0.0 ± 9.1	-	34.9 ± 1.8	40.4 ± 4.1**	70.2 ± 2.3**	34.2
(**13**) *Achras sapota*	Water	0.0 ± 9.1	-	22.0 ± 1.8	40.8 ± 0.9**	65.6 ± 2.8**	45.4
(**14**) *Achras sapota*	EtOH	0.0 ± 9.1	-	35.8 ± 2.9	36.7 ± 3.1**	62.8 ± 5.4**	62.0
(**15**) *Citrus reticulata*	Water	0.0 ± 9.1	-	-29.4 ± 2.4	13.8 ± 5.6	53.2 ± 5.0**	88.4
(**16**) *Citrus reticulata*	EtOH	0.0 ± 9.1	-	33.0 ± 3.1	45.9 ± 2.3	70.6 ± 1.3**	31.3
(**17**) *Citrus maxima*	Water	0.0 ± 9.1	-	19.3 ± 2.0	38.5 ± 2.3**	50.9 ± 6.6**	85.0
(**18**) *Citrus maxima*	EtOH	0.0 ± 9.1	-	32.1 ± 2.7	32.6 ± 3.5**	61.9 ± 2.7**	68.6
Indomethacin	-	0.0 ± 3.6	14.5 ± 2.7	30.2 ± 1.6**	47.6 ± 2.3**	80.3 ± 1.5**	25.0 μM (8.9 μg/ml)

[a]Each value represents mean ± S.E.M. of four determinations. Statistical significance, * $p<0.05$, ** $p<0.01$ compared to the control group (0 μg/ml of sample).

(-) = not determined.

Table 3. Inhibitory activity on PGE_2 production[a] of Thai fruits

Sample	% Inhibition at various concentrations (μg/ml)							IC_{50} (μg/ml)
	Solvent	0	1	3	10	30	100	
(**1**) *Nephelium lappaceum*	Water	0.0 ± 0.7	-	-	-	-	22.0 ± 1.0**	>100
(**2**) *Nephelium lappaceum*	EtOH	0.0 ± 0.7	-	-	-	-	18.7 ± 0.9**	>100
(**3**) *Syzygium aquem*	Water	0.0 ± 0.7	-	-	-	-	21.3 ± 1.3**	>100
(**4**) *Syzygium aquem*	EtOH	0.0 ± 0.7	-	-	-	-	22.7 ± 1.0**	>100
(**5**) *Garcinia mangostana*	Water	0.0 ± 0.7	-	-	-	-	21.5 ± 0.6**	>100
(**6**) *Garcinia mangostana*	EtOH	0.0 ± 0.7	-	-	-	-	21.4 ± 0.8**	>100
(**7**) *Aglaia dookkoo*	Water	0.0 ± 0.7	-	-	-	-	17.3 ± 0.2**	>100
(**8**) *Aglaia dookkoo*	EtOH	0.0 ± 0.7	-	-	-	-	23.2 ± 0.6**	>100
(**9**) *Guava sativa*	Water	0.0 ± 0.7	-	-	-	-	25.4 ± 0.9**	>100
(**10**) *Guava sativa*	EtOH	0.0 ± 0.7	-	-	-	-	24.3 ± 0.5**	>100
(**11**) *Carica papaya*	Water	0.0 ± 0.7	-	-	-	-	25.7 ± 0.6**	>100
(**12**) *Carica papaya*	EtOH	0.0 ± 0.7	-	-	-	-	25.4 ± 0.8**	>100
(**13**) *Achras sapota*	Water	0.0 ± 0.7	-	-	-	-	22.0 ± 1.2**	>100
(**14**) *Achras sapota*	EtOH	0.0 ± 0.7	-	-	-	-	21.0 ± 1.0**	>100
(**15**) *Citrus reticulata*	Water	0.0 ± 0.7	-	-	-	-	14.7 ± 2.4**	>100
(**16**) *Citrus reticulata*	EtOH	0.0 ± 0.7	-	-	-	-	20.2 ± 0.6**	>100
(**17**) *Citrus maxima*	Water	0.0 ± 0.7	-	-	-	-	22.2 ± 1.0**	>100
(**18**) *Citrus maxima*	EtOH	0.0 ± 0.7	-	-	-	-	25.6 ± 0.3**	>100
Indomethacin		0.0 ± 2.0	52.7 ± 0.7**	74.6 ± 0.1**	76.9 ± 0.3**	77.2 ± 0.1**	78.1 ± 0.2**	0.4 μM (0.14 μg/ml)

[a]Each value represents mean ± S.E.M. of four determinations. (-) = not determined.

Statistical significance, * $p<0.05$, ** $p<0.01$ compared to the control group (0 μg/ml of sample).

Table 4. Anti-allergic activity on β-hexosaminidase release[a] from RBL-2H3 cells of Thai fruits

Sample	% Inhibition at various concentrations (μg/ml)						IC_{50} (μg/ml)
	Solvent	0	3	10	30	100	
(**1**) *Nephelium lappaceum*	Water	0.0 ± 8.2	-	39.6± 2.4*	60.0 ± 2.5**	78.9 ± 4.2**	17.7
(**2**) *Nephelium lappaceum*	EtOH	0.0 ± 8.3	-	1.0 ± 4.6	53.7 ± 6.2**	79.1 ± 6.5**	36.4
(**3**) *Syzygium aquem*	Water	0.0 ± 8.2	-	-16.5 ± 6.4	29.4 ± 5.1	75.5 ± 6.8**	51.9
(**4**) *Syzygium aquem*	EtOH	0.0 ± 8.3	-	39.8 ± 6.9*	47.8 ± 5.6**	82.0 ± 4.8**	21.8
(**5**) *Garcinia mangostana*	Water	0.0 ± 8.2	-	42.5 ± 7.1*	67.4 ± 1.4**	74.4 ± 2.1**	13.5
(**6**) *Garcinia mangostana*	EtOH	0.0 ± 8.3	-	40.2 ± 4.9*	55.0 ± 6.0**	99.6 ± 3.9**	17.4
(**7**) *Aglaia dookkoo*	Water	0.0 ± 8.2	-	-	-	26.1 ± 4.2**	>100
(**8**) *Aglaia dookkoo*	EtOH	0.0 ± 8.3	-	-	-	49.0 ± 1.2**	>100
(**9**) *Guava sativa*	Water	0.0 ± 10.0	-	-	-	45.2 ± 6.3**	>100
(**10**) *Guava sativa*	EtOH	0.0 ± 6.7	-	-	-	49.7 ± 3.6**	>100
(**11**) *Carica papaya*	Water	0.0 ± 10.0	-	39.5 ± 0.7	49.2 ± 2.5**	61.7 ± 5.2**	30.6
(**12**) *Carica papaya*	EtOH	0.0 ± 6.7	-	-	-	37.6 ± 4.9**	>100
(**13**) *Achras sapota*	Water	0.0 ± 9.3	-	-1.9 ± 3.3	30.1 ± 3.5**	57.5 ± 8.0**	71.4
(**14**) *Achras sapota*	EtOH	0.0 ± 6.7	-	-	-	40.4 ± 4.4**	>100
(**15**) *Citrus reticulata*	Water	0.0 ± 6.7	-	-	-	12.9 ± 5.4	>100
(**16**) *Citrus reticulata*	EtOH	0.0 ± 6.7	-	-	-	22.3 ± 4.4	>100
(**17**) *Citrus maxima*	Water	0.0 ± 6.7	-	-	-	38.0 ± 6.0*	>100
(**18**) *Citrus maxima*	EtOH	0.0 ± 6.7	-	-	-	38.3 ± 7.0**	>100
Ketotifen fumarate	-	0.0 ± 5.9	-	12.8 ± 0.5	38.3 ± 3.2 **	68.2 ± 1.5**	47.5 μM (20.2[b])

[a]Each value represents mean ± S.E.M. of four determinations. (-) = not determined.

Statistical significance, * $p<0.05$, ** $p<0.01$ compared to the control group (0 μg/ml of sample). [b]Value in parenthesis is IC_{50} (μg/ml).

Table 5. Inhibitory activity on TNF-α production[a] of Thai fruits using RBL-2H3 cells

Sample	% Inhibition at various concentrations (μg/ml)						IC_{50} (μg/ml)
	Solvent	0	3	10	30	100	
(**1**) *Nephelium lappaceum*	Water	0.0 ± 12.3	-	-	-	4.3 ± 7.8	>100
(**2**) *Nephelium lappaceum*	EtOH	0.0 ± 9.6	-	-	-	46.3 ± 6.8*	>100
(**3**) *Syzygium aquem*	Water	0.0 ± 12.3	-	-	-	41.8 ± 9.7*	>100
(**4**) *Syzygium aquem*	EtOH	0.0 ± 9.6	-	-	-	35.2 ± 8.2*	>100
(**5**) *Garcinia mangostana*	Water	0.0 ± 12.3	-	-	-	41.5 ± 8.4*	>100
(**6**) *Garcinia mangostana*	EtOH	0.0 ± 9.6	-	-	-	32.6 ± 6.5*	>100
(**7**) *Aglaia dookkoo*	Water	0.0 ± 12.3	-	-	-	14.6 ± 3.0	>100
(**8**) *Aglaia dookkoo*	EtOH	0.0 ± 9.6	-	-	-	37.3 ± 2.3*	>100
(**9**) *Guava sativa*	Water	0.0 ± 7.3	-	-	-	27.6 ± 6.6*	>100
(**10**) *Guava sativa*	EtOH	0.0 ± 4.5	-	-	-	-17.9 ± 10.5	>100
(**11**) *Carica papaya*	Water	0.0 ± 7.3	-	-	-	18.4 ± 3.4*	>100
(**12**) *Carica papaya*	EtOH	0.0 ± 4.5	-	-	-	28.3 ± 2.1**	>100
(**13**) *Achras sapota*	Water	0.0 ± 7.3	-	12.5 ± 2.0	31.3 ± 3.4*	50.9 ± 5.3**	93.8
(**14**) *Achras sapota*	EtOH	0.0 ± 4.5	-	-	-	17.3 ± 2.4*	>100
(**15**) *Citrus reticulata*	Water	0.0 ± 7.3	-	-	-	30.0 ± 6.1**	>100
(**16**) *Citrus reticulata*	EtOH	0.0 ± 4.5	-	-	-	27.5 ± 1.5*	>100
(**17**) *Citrus maxima*	Water	0.0 ± 7.3	-	-	-	3.8 ± 6.1	>100
(**18**) *Citrus maxima*	EtOH	0.0 ± 4.5	-	-	-	20.7 ± 2.4**	>100

[a]Each value represents mean ± S.E.M. of four determinations.

Statistical significance, * $p<0.05$, ** $p<0.01$ compared to the control group (0 μg/ml of sample).

(-) = not determined.

Output from this research work

The present study may support the use of Thai fruits for treatment of inflammation and allergy.

APPENDIX