



Antibacterial, anti-inflammatory and anti-allergic activities of standardised pomegranate rind extract

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ABSTRACT

Antibacterial, anti-inflammatory and anti-allergic activities of standardised pomegranate rind extract (SPRE) containing 13% w/w ellagic acid were studied *in vitro*. The antibacterial activity of SPRE was determined using the disc diffusion and broth microdilution methods. SPRE exhibited a potent bacteriostatic effect against *Propionibacterium acnes*, a Gram-positive anaerobe, with a MIC of 15.6 µg/ml, and Gram-positive facultative anaerobic bacteria, *Staphylococcus aureus* and *Staphylococcus epidermidis*, with MICs of 7.8–15.6 µg/ml. Anti-inflammatory activity of SPRE was evaluated by measuring the inhibition of nitric oxide (NO) production by murine macrophage-like RAW264.7 cells. SPRE exhibited a potent NO inhibitory effect, with an IC₅₀ of 10.7 µg/ml. Evaluation of the anti-allergic activity showed that SPRE inhibited the release of β-hexosaminidase from antigen-stimulated rat basophilic leukemia (RBL-2H3) cells with an IC₅₀ of 20.9 µg/ml. In addition, SPRE exhibited only moderate cytotoxicity on human keratinocyte cells, with CC₅₀ of 33.6 µg/ml. These findings support the potential use of SPRE as a nutraceutical for antibacterial, anti-inflammatory and anti-allergic proposes.

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1. Introduction

Pomegranate (*Punica granatum* L.) fruits are widely consumed, fresh and in commercial products, such as juices, jams and wines. Pomegranate rind is a rich source of hydrolysable tannins of the ellagitannin group. Pomegranate rind extracts have recently attracted interest because of their potential use as natural food preservatives and nutraceuticals (Negi, Jayaprakasha, & Jena, 2003). The crude methanol extract of the rind has been reported to contain 192 mg/g of total phenolics (Surveswaran, Cai, Corke, & Sun, 2007). The extract showed high antioxidant activity, with trolox equivalent antioxidant capacity (TEAC) values of 395 and 316 mmol/100 g dry weight, as evaluated by 1,1-diphenyl-2-picrylhydrazyl (DPPH[•]) and 2,2'-azinobis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS^{•+}) assays, respectively. In a recent study we have shown that ellagic acid (Fig. 1) is the major antioxidative constituent in the methanol extract of pomegranate rind. We have used this compound as an indicative marker for standardisation of pomegranate rind extract (Panichayupakaranant, Issuriya, Sirikatitham, & Wang, *in press*). The detailed methodology for

the preparation and standardisation of ellagic acid-rich pomegranate extracts has also been recently established (Panichayupakaranant, Itsuriya, & Sirikatitham, 2010).

Ellagitannins isolated from pomegranate have been reported to exhibit antibacterial activity against both methicillin-resistant and methicillin-sensitive *Staphylococcus aureus*, with MICs of 62.5 µg/ml (Machado et al., 2003). Pomegranate juice and peel have also been shown to possess anticancer activities (Romier-Crouzet et al., *in press*). This may be associated with plant-based anti-inflammatory effects. Pomegranate extract exhibited anti-inflammatory activity via inhibition of NF-κB activity and Erk1/2 activation and decreased NO and PGE₂ synthesis in human intestinal Caco-2 cells. In addition, ellagic acid was capable of decreasing NF-κB activation through a mechanism independent of IκBα phosphorylation (Romier, Van De Walle, During, Larondelle, & Schneider, 2008). Thus, pomegranate extracts could be particularly promising in dietary prevention of intestinal inflammation.

In order to investigate the full potential of standardised pomegranate rind extract (SPRE) as a natural food preservative and nutraceutical, we now report the antibacterial activity of SPRE. Additionally, we also report herein the inhibitory effect on NO production by murine macrophage-like RAW264.7 cells, and the inhibitory effect on β-hexosaminidase release from antigen-stimulated rat basophilic leukemia (RBL-2H3). The cytotoxicity of SPRE was evaluated against human keratinocyte cells.

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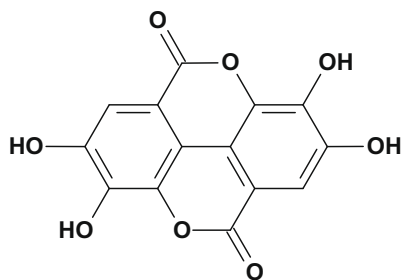


Fig. 1. Structure of ellagic acid.

2. Materials and methods

2.1. Chemicals and reagents

Standard ellagic acid and norfloxacin were purchased from Fluka (Switzerland). Methanol (HPLC and analytical grades) and ethyl acetate (analytical grade) were purchased from Labscan Asia (Thailand). Acetic acid was from J.T. Baker (USA). Water was purified in a Milli-Q system (Millipore, Bedford, MA, USA). Tetracycline hydrochloride, lipopolysaccharide (LPS, from *Escherichia coli*), RPMI-1640 medium, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT), L-nitroarginine (L-NA), caffeic acid phenethyl ester (CAPE), phosphate buffered saline (PBS), minimum essential eagle medium (MEM) and anti-DNP-IgE (monoclonal anti-DNP) were purchased from Sigma–Aldrich (Sigma–Aldrich, Missouri, USA). Foetal calf serum (FCS), trypan blue and trypsin were from Gibco (USA). Penicillin–streptomycin was purchased from Invitrogen (USA). Dinitrophenylated bovine serum albumin was prepared as described previously (Tada & Okumura, 1971). Standard tetracycline and norfloxacin discs were from Oxoid (UK).

2.2. Plant material

Pomegranate fruits were collected from Mengzhi pomegranate garden, Yunnan, China, in August, 2006. A voucher specimen (specimen No. SKP 158 16 07 01) was identified by Associate Professor Pharkphoom Panichayupakaranant, and has been deposited at the herbarium of the Faculty of Pharmaceutical Sciences, Prince of Songkla University, Thailand. Pomegranate rinds were dried at 50 °C for 24 h in a hot air oven and were reduced to powder using a grinder and a sieve No. 45.

2.3. Preparation of SPRE

SPRE was prepared and standardised to contain 13% w/w ellagic acid by previously described methods (Panichayupakaranant et al., 2010). Briefly, the dried powder of pomegranate rind (0.5 kg) was extracted twice with 2 l of methanol containing 10% (v/v) water under reflux conditions for 1 h. The pooled extracts were dried *in vacuo*. The crude extract was then suspended in 2% aqueous acetic acid and partitioned with ethyl acetate. The pooled ethyl acetate fractions were then evaporated to dryness *in vacuo*.

2.4. Microorganisms and media

Propionibacterium acnes (DMST 14916), *Salmonella typhimurium* (DMST 562) and *Shigella sonnei* (DMST 561) were obtained from the Department of Medical Science Center, Thailand. *S. aureus* (ATCC 25923), *S. aureus* (isolated strains PSU01–PSU07), *Staphylococcus epidermidis* (ATCC 14990), *Salmonella typhi*, and *E. coli* (ATCC 25922) were obtained from the Department of Microbiology, Fac-

ulty of Medicine, Prince of Songkla University. Mueller–Hinton agar and Mueller–Hinton broth were purchased from Merck.

2.5. Antibacterial susceptibility testing

These experiments were performed by the disc diffusion method (NCCLS, 2008) with some modification. *P. acnes* was incubated in Mueller–Hinton agar with 5% blood for 72 h under anaerobic conditions, and volume adjusted with 0.85% sodium chloride to yield approximately 10^8 CFU/ml compared to the turbidity of McFarland No. 0.5. Molten Mueller–Hinton agar, with 5% blood, was used as an agar base. The prepared inoculum was streaked onto the surface of the agar base with a cotton stick. A sterile paper disc ($\varnothing$ 6 mm) was impregnated with 10 μ l of plant extract (200 mg/ml) and the disc was placed on the agar. The concentration of each plant extract was 2 mg/disc. DMSO was used as a negative control, while standard tetracycline hydrochloride (30 μ g/disc) and norfloxacin (10 μ g/disc) discs were used as positive controls. Plates were then incubated at 37 °C for 72 h under anaerobic conditions.

The aerobic and facultative anaerobic bacteria were incubated in Mueller–Hinton agar for 24 h at 37 °C and adjusted to yield approximately 10^8 CFU/ml. Subsequent procedures were the same as those described above except that the plates were incubated at 37 °C for 24 h under defined conditions.

All disc diffusion tests were performed in triplicate and antibacterial activity was expressed as the mean of inhibition zones (mm).

2.6. Determination of minimum inhibitory and bactericidal concentration

The minimum inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) were determined by the microdilution assay (NCCLS, 2008). Cultures of *P. acnes* were prepared as 72 h broth cultures, whereas cultures of aerobic and facultative anaerobic bacteria were 24 h broth cultures. The MIC was defined as the lowest concentration of the compound to inhibit the growth of microorganisms, and the MBC was defined as the lowest concentration of the compound to kill the microorganisms.

2.7. Assay for NO inhibitory effect using RAW264.7 cells

Inhibitory effect on nitric oxide (NO) production by murine macrophage-like RAW264.7 cells was evaluated using a method modified from that previously reported (Banskota et al., 2003). 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 μ g/ml) and 10% FCS. The cells were harvested with trypsin–EDTA and diluted to a suspension in fresh medium. The cells were seeded in 96-well plates with 1×10^5 cells/well and allowed to adhere for 1 h at 37 °C in a humidified atmosphere containing 5% CO₂. The medium was then replaced with fresh medium containing 100 μ g/ml of LPS, together with the test samples at various concentrations (3–100 μ g/ml for extracts and 3–100 μ M for pure compounds), and then incubated at 37 °C for 48 h.

NO production was determined by measuring the accumulation of nitrite in the culture supernatant using the Griess reagent. Cytotoxicity was determined using the MTT colorimetric method. Briefly, after the 48 h incubation period with the test samples, MTT solution (10 μ l, 5 mg/ml in PBS) was added to the wells. After further incubation (4 h), the medium was removed, and isopropanol containing 0.04 M HCl was added to dissolve the formazan produced 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 if the optical density of the

sample-treated group was less than 80% of that in the control (vehicle-treated) group. L-NA (NO synthase inhibitor) and CAPE (NF- κ B inhibitor) were used as positive controls. 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 IC₅₀ values were determined graphically ($n = 4$):

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

A – C: NO₂[−] concentration (μM) [A: LPS (+), sample (−); B: LPS (+), sample (+); C: LPS (−), sample (−)].

2.8. Inhibitory effects 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) were evaluated by the following modified method (Matsuda, Tewtrakul, Morikawa, Nakamura, & Yoshikawa, 2004). Briefly, RBL-2H3 cells were dispensed in 24-well plates at a concentration of 2×10^5 cells/well using minimum essential eagle medium (MEM) containing 10% foetal 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% CO₂ 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 MgCl₂, 1 mM CaCl₂, 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. Aliquots (20 μl) of test sample solutions were then added to each well and the plate incubated for 10 min. This was followed by addition of 20 μl of antigen (DNP-BSA, final concentration is 10 μg/ml) and further incubation at 37 °C for 20 min to stimulate the cells to degranulate. The supernatants were 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 reactions were stopped by adding 200 μl of stop solution (0.1 M Na₂CO₃/NaHCO₃, pH 10.0). The absorbances were 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%). The inhibition (%) of the release of β-hexosaminidase by the test samples was calculated by the following equation, and IC₅₀ 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 (−).

2.9. In vitro assay for cytotoxic activity

Cytotoxicity assay was performed using primary human keratinocytes (GibcoTM) (Skehan et al., 1990). The cells were maintained as a monolayer culture in defined keratinocyte-SFM in a 5% CO₂ humidified incubator at 37 °C and passaged in 25 cm² tissue culture flasks. The cell cultures were subcultured weekly using 0.05% trypsin–EDTA to harvest the cells.

Cells were harvested from monolayers and centrifuged at 1000g for 10 min and resuspended in medium to make a single cell suspension. Viability of the cells was determined by trypan blue exclusion. The cell concentration was counted in a hemocytometer and the cells were then diluted to give the optimal seeding density (3000 cells/well) and 0.1 ml of the cell suspension

was plated into the wells of 96-well microtitre plates. Plates containing cells were incubated for 24 h before the addition of test sample. The test sample, at twice the required final concentration, was added (0.1 ml) to six wells. Sequential twofold dilutions were then performed to produce five concentrations (250, 125, 62.5, 31.3, 15.6 μg/ml). The final dilution used for treating the cells contained not more than 1% of the initial solvent (DMSO), this concentration being used in the solvent control wells. Culture was then continued for 6 days. At the end of the exposure time, cell growth was analysed using the SRB assay. The cytotoxic concentration that caused the reduction of viable cells by 50% (CC₅₀) was calculated from probit analysis (SPSS Version 10).

3. Results and discussion

Antibacterial susceptibility determination of SPRE using the disc diffusion method revealed that SPRE, at concentration of 2 mg/disc, possessed an inhibitory effect against the Gram-positive bacteria including *P. acnes*, *S. aureus* (both standard and isolated strains) and *S. epidermidis*. In contrast, an inhibitory effect was not found against Gram-negative bacteria, including *S. typhimurium*, *S. typhi*, *S. sonnei* and *E. coli* (Table 1). Although it has been reported that the pomegranate root extract possessed inhibitory activity against Gram-negative bacteria, such as *S. sonnei*, *S. typhi*, *Vibrio cholera* and *E. coli* (Mathabe, Nikolova, Lall, & Nyazema, 2006), the fruit rind extract was not active. Results obtained from our study are in agreement with those of Ahmad and Beg (2001) who reported that alcohol extracts of pomegranate fruits showed antibacterial activity against *S. aureus*, but were not active against *E. coli*, *Shigella paratyphi* and *Shigella dysentery*.

In addition, it has been reported that ellagitannins isolated from pomegranate exhibited antibacterial activity against both methicillin-resistant and methicillin-sensitive *S. aureus* (Machado et al., 2003). However, our findings indicate that all tested bacteria were resistant to ellagic acid at concentrations up to 2 mg/ml. The potency of SPRE against *P. acnes*, *S. aureus* and *S. epidermidis* was subsequently evaluated by determination of MIC and MBC values. The results showed that SPRE exhibited a satisfactory inhibitory effect on the Gram-positive anaerobe *P. acnes*, with MIC value of 15.6 μg/ml. The Gram-positive facultative anaerobic bacteria, *S. aureus* and *S. epidermidis* had MIC values of 7.8–15.6 and 7.8 μg/ml, respectively (Table 2). However, MBC values of SPRE against these bacteria were much higher than the MIC values. It implies that SPRE possesses only bacteriostatic activity against the three bacteria.

Table 1
Inhibition zones of standardised pomegranate rind extract (SPRE).

Microorganisms	Inhibition zone (mm ± SD)		
	SPRE	Tetracycline	Norfloxacin
<i>P. acnes</i> (DMST 14916)	21.5 ± 1.89	60.3 ± 0.61	–
<i>S. epidermidis</i> (ATCC 14990)	19.1 ± 0.49	33.5 ± 0.11	–
<i>S. aureus</i> (ATCC 25923)	18.6 ± 0.38	32.5 ± 0.54	–
<i>S. aureus</i> (PSU01)	18.8 ± 0.40	33.4 ± 0.62	–
<i>S. aureus</i> (PSU02)	18.1 ± 0.42	31.8 ± 0.53	–
<i>S. aureus</i> (PSU03)	16.8 ± 0.38	31.5 ± 0.45	–
<i>S. aureus</i> (PSU04)	19.4 ± 0.30	32.2 ± 0.53	–
<i>S. aureus</i> (PSU05)	18.6 ± 0.79	31.2 ± 0.70	–
<i>S. aureus</i> (PSU06)	15.2 ± 0.60	30.3 ± 0.50	–
<i>S. aureus</i> (PSU07)	16.2 ± 0.78	29.5 ± 0.81	–
<i>S. typhimurium</i> (DMST 562)	R	–	24.5 ± 0.50
<i>S. typhi</i>	R	–	25.0 ± 0.45
<i>S. sonnei</i> (DMST 561)	R	–	39.0 ± 0.65
<i>E. coli</i> (ATCC 25922)	R	–	29.0 ± 0.52

R, resistant; (–), not performed. Data are means ± SD ($n = 3$).

Table 2

Minimum inhibitory concentration and minimum bactericidal concentration of standardised pomegranate rind extract (SPRE) against *P. acnes*, *S. epidermidis* and *S. aureus*.

Microorganisms	MIC ($\mu\text{g/ml}$)		MBC ($\mu\text{g/ml}$)	
	SPRE	Tetracycline	SPRE	Tetracycline
<i>P. acnes</i> (DMST 14916)	15.6	0.03	>1000	>30
<i>S. epidermidis</i> (ATCC 14990)	7.81	0.23	250	>30
<i>S. aureus</i> (ATCC 25923)	7.81	0.47	500	>30
<i>S. aureus</i> (PSU01)	7.81	0.23	500	>30
<i>S. aureus</i> (PSU02)	7.81	1.87	1000	>30
<i>S. aureus</i> (PSU03)	15.6	30	1000	>30
<i>S. aureus</i> (PSU04)	7.81	0.47	1000	>30
<i>S. aureus</i> (PSU05)	15.6	3.75	1000	>30
<i>S. aureus</i> (PSU06)	15.6	0.47	1000	>30
<i>S. aureus</i> (PSU07)	7.81	0.47	1000	>30

Table 3

Nitric oxide inhibitory activities of ellagic acid and standardised pomegranate rind extract (SPRE).

Compounds	IC ₅₀ $\pm$ SEM ($\mu\text{g/ml}$)
SPRE	10.7 $\pm$ 1.3
Ellagic acid	1.9 $\pm$ 0.7
L-Nitroarginine	13.5 $\pm$ 0.4
Caffeic acid phenethylster	1.4 $\pm$ 0.3

Data are means $\pm$ SEM ($n = 3$).

Table 4

Anti-allergic activities of ellagic acid and standardised pomegranate rind extract (SPRE).

Compounds	IC ₅₀ $\pm$ SEM ($\mu\text{g/ml}$)
SPRE	20.9 $\pm$ 2.0
Ellagic acid	4.3 $\pm$ 0.9
Ketotifen fumarate	20.2 $\pm$ 0.4

Data are means $\pm$ SEM ($n = 3$).

Our findings also suggest that the anti-diarrhoeal effect of pomegranate rind may be due to its inhibitory effect on Gram-positive bacteria, as well as the astringent effect of tannins. It has also been suggested that pomegranate extract reduces diarrhoea by inhibiting gastrointestinal motility and PGE₂-induced enteropooling (Das et al., 1999).

Evaluation of inhibitory activity against NO production revealed that SPRE possessed marked anti-NO effects, with an IC₅₀ value of 10.7 $\mu\text{g/ml}$. Its potency was the same as that of L-nitroarginine, a NO synthase inhibitor (Table 3). However, the potency of SPRE was about fivefold lower than those of ellagic acid and caffeic acid phenethylster. Our finding is in agreement with previous reports on anti-inflammatory activity of pomegranate extract (Romier-Crouzet et al., 2009) and ellagic acid (Romier et al., 2008).

Determination of anti-allergic activity using inhibitory assay, on the release of β -hexosaminidase from RBL-2H3 cells, revealed that SPRE and ellagic acid possessed marked inhibitory activity with IC₅₀ values of 20.9 and 4.3 $\mu\text{g/ml}$, respectively. Therefore, both SPRE and ellagic are the potent anti-allergic agents when com-

pared to the inhibitory effect of ketotifen fumarate, a positive control (Table 4). In addition, SPRE exhibited moderate cytotoxicity in human keratinocyte cells with CC₅₀ 33.6 $\pm$ 1.27 $\mu\text{g/ml}$. We conclude that toxic effects of SPRE occurred at higher doses than those effective therapeutic doses. This result is similar to that reported by Vidal et al. (2003).

The results from this study support the potential use of SPRE as a nutraceutical for antibacterial, anti-inflammatory and anti-allergic proposes. However, the use of SPRE as a natural preservative is not recommended, due to its narrow spectrum antibacterial activity.

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References

- Ahmad, I., & Beg, A. Z. (2001). Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multi-drug resistant human pathogens. *Journal of Ethnopharmacology*, 74, 113–123.
- Banskota, A. H., Tezuka, Y., Nguyen, N. T., Awale, S., Nobukawa, T., & Kadota, S. (2003). DPPH radical scavenging and nitric oxide inhibitory activities of the constituents from the wood of *Taxus yunnanensis*. *Planta Medica*, 69, 500–505.
- Das, A. K., Mandal, S. C., Banerjee, S. K., Sinha, S., Das, J., Saha, B. P., et al. (1999). Studies on antidiarrhoeal activity of *Punica granatum* seed extract in rats. *Journal of Ethnopharmacology*, 68, 205–208.
- Machado, T. B., Pinto, A. V., Pinto, M. C., Leal, I. C., Silva, M. G., Amaral, A. C., et al. (2003). In vitro activity of Brazilian medicinal plants, naturally occurring naphthoquinones and their analogues, against methicillin-resistant *Staphylococcus aureus*. *International Journal of Antimicrobial Agents*, 21, 279–284.
- Mathabe, M. C., Nikolova, R. V., Lall, N., & Nyazema, N. Z. (2006). Antibacterial activities of medicinal plants used for the treatment of diarrhoea in Limpopo Province, South Africa. *Journal of Ethnopharmacology*, 105, 286–293.
- Matsuda, H., Tewtrakul, S., Morikawa, T., Nakamura, A., & 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 and Medicinal Chemistry*, 12, 5891–5898.
- NCCLS (2008). *Performance standards for antimicrobial susceptibility testing; Ninth informational supplement*, NCCLS document M100-S9. (pp. 120–126). Wayne: National Committee for Clinical Laboratory Standard.
- Negi, P. S., Jayaprakasha, G. K., & Jena, B. S. (2003). Antioxidant and antimutagenic activities of pomegranate peel extracts. *Food Chemistry*, 80, 393–397.
- Panichayupakaranant, P., Issuriya, A., Sirikittham, A., & Wang, W. (in press). Antioxidant assay-guided purification and liquid chromatographic determination of ellagic acid in pomegranate peel. *Journal of Chromatographic Science*.
- Panichayupakaranant, P., Itsuriya, A., & Sirikittham, A. (2010). Preparation method and stability of ellagic acid-rich pomegranate fruit peel extracts. *Pharmaceutical Biology*, 48, 201–205.
- Romier, B., Van De Walle, J., During, A., Larondelle, Y., & Schneider, Y. J. (2008). Modulation of signaling NF- κ B activation pathway by polyphenols in human intestinal Caco-2 cells. *British Journal of Nutrition*, 100, 542–551.
- Romier-Crouzet, B., Van De Walle, J., During, A., Joly, A., Rousseau, C., Henry, O., et al. (2009). Inhibition of inflammatory mediators by polyphenolic plant extracts in human intestinal Caco-2 cells. *Food and Chemical Toxicology*, 47, 1221–1230.
- Skehan, P., Storeng, R., Scudiero, D., Monks, A., McMahon, J., Vistica, D., et al. (1990). New colorimetric cytotoxicity assay for anticancer-drug screening. *Journal of the National Cancer Institute*, 82, 1107–1112.
- Surveswaran, S., Cai, Y., Corke, H., & Sun, M. (2007). Systematic evaluation of natural antioxidants from 133 Indian medicinal plants. *Food Chemistry*, 102, 938–953.
- Tada, T., & Okumura, K. (1971). Regulation of homocytotropic antibody formation in the rat I. Feed-back regulation of passively administered antibody. *The Journal of Immunology*, 106, 1002–1011.
- Vidal, A., Fallarero, B. R., Peña, M. E., Medica, B., Gra, F., Rivera, Y., et al. (2003). Studies on the toxicity of *Punica granatum* L. (Punicaceae) whole fruit extracts. *Journal of Ethnopharmacology*, 89, 295–300.