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Antioxidant Activity and Bioefficacy of Pomegranate *Punica granatum* Linn. Peel and Seed Extracts

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Abstract: Pomegranate (*Punica granatum* Linn.) peel and seed ethanolic and water extracts (PPE/e, PPE/w, PSE/e, PSE/w) were quantified for total phenolic compounds (TPC) and flavonoids content (FC) and antioxidant cytotoxic and antiproliferation activities. TPC and FC ranged as PPE/e > PPE/w > PSE/e > PSE/w. The amount of TPCs were 449.60±4.40, 380.54±5.87, 77.93±1.62 and 51.58±0.85 µg GAE/mg and of FCs were 38.44±1.44, 26.06±0.93, 16.66±0.47 and 10.55±0.14 µg CAE/mg respectively. The free radical scavenging activity determined by DPPH radicals and the lipid peroxidation inhibition were as high as AA, CA and EGCG the antioxidant controls. In particularly, PPE/e possessed highest free radical scavenging, IC_{50} of 121.65±2.66 µg/ml and lipid peroxidation inhibition activity, IC50 18.04±1.95 µg/ml. All pomegranate extracts were not toxic to normal cells. The cytotoxicity of the extracts ranged as PPE/e > PSE/w > PPE/w > PSE/e with LC_{50} values at 24 hrs of 1,206.98±12.73, 1,294.88±61.28, 1,743.31±20.17 and 2,375.28±69.54 µg/ml, respectively. TPC and FC of the extracts were well correlated to the antioxidant activities, but not to the cytotoxicity. PPE/e and PPE/w most potentially inhibited the proliferation of MCF-7 cells with LC_{50} of 375.75±1.22 and 471.80±4.37 µg/ml. The statistically significant NOAEL ranged as PPE/w > PSE/e > PPE/e > PSE/w with values of 1,250, 1,000, 750 and 100 µg/ml respectively. LOAEL was PPE/w = PSE/e > PPE/e > PSE/w values of 1,500, 1,000 and 500 µg/ml respectively. MOS and TI values were PPE/w > PSE/e > PPE/e > PSE/w. NOAEL, LOAEL. MOS and TI data provide further study for the pharmacological and therapeutic development of pomegranate products.

Key words: Antioxidant Activity · Lipid Peroxidation Inhibition · Cytotoxicity · Antiproliferation · NOAEL · LAOEL

INTRODUCTION

Pomegranate *Punica granatum* Linn. has been known for traditional uses to remedy a numbers of symptoms, such as its leaves for eye sore; flowers for blood clotting; rind and dried fruit for diarrhea; stem and root bark for ridding of parasites; and seeds for scurvy. It was also reported to be used in modern medicine. The peel extract was a potent virucidal agent [1] against genital herpes virus [2] due to tannins. It was also used to treat the infection of male or female sexual organs, mastitis, acne, folliculitis, pile, allergic dermatitis and dysentery [3]. Pomegranate peel and seed possessed potent antioxidant properties [4, 5]. Polyphenols from pomegranate fermented juice, peel and seed oil [6] and ethanolic juice [7] were found synergistically inhibited the proliferation and induced apoptosis of human prostate cancer cells. Some evidence of antioxidants and anticancer of pomegranate are available. However, there is no information on toxicological profile of the pomegranate products. This study thus aimed to investigate the potential of pomegranate peel and seed extracts on antioxidant activity, toxicological property and antiproliferative effect on MCF-7, the human breast adenocarcinoma cell line.

As a broad spectral basis of pharmacological action and toxicity of phytochemicals is needed for predicting the adverse effects on human beings and developing

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drugs [8]. The information on cytotoxicity of pomegranate extracts was then statistically identified for no-observed-adverse-effect-level (NOAEL), lowest-observed-adverse-effect-level (LOAEL), margin of safety (MOS) and therapeutic index (TI). Thus, the information and knowledge from this study will be useful for further research on pomegranate products for pharmacological development and cancer therapy.

MATERIALS AND METHODS

Plant Material and Extract Preparation: Pomegranate fruits were collected from local farms in Klangdong, Saraburi, Thailand. The plant was taxonomically identified by the Royal Forest Department of Thailand, specimen voucher no. 080252. The fruits were cleaned. The peel and the seeds were separated, dried and ground to powder and stored. The peel and the seed powders of 50 g was extracted in 500 ml of 70% ethanol or water for 24 hrs in a Soxhlet extraction apparatus, evaporated, lyophilized and kept at -20C for further use.

Chemicals: Folin-Ciocalteau reagent and gallic acid were obtained from Fluka Chemie AG, Buchs, Switzerland. Ascorbic acid (AA), 3-*tert*-butyl-4-hydroxyanisole (BHA), catechin (CA), epigallocatechin-3-gallate (EGCG) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) were purchased from Sigma Chemical Company, St. Louis, MO, USA. Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12 (DMEM/F-12), fetal bovine serum (FBS) and penicillin/streptomycin were from GIBCO, Invitrogen Corporation, NY, USA. 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) was purchased from Molecular Probes, Invitrogen Corporation, NY, USA. All other chemicals and reagents were of analytical grade.

Total Phenolic Compounds Measurement: Total phenolic compounds (TPC) were quantified by Folin-Ciocalteu method [9]. One hundred microliters of sample, dissolved in 70% ethanol or water, were mixed with 2 ml of 2% sodium carbonate solution containing 100 µl Folin-Ciocalteu reagent (Folin-Ciocalteu: methanol, 1:1, v/v) and incubated for 30 min. The optical absorbance was measured at 760 nm. TPC content was expressed as micrograms of gallic acid equivalents (GAE) per milligrams dried extract.

Flavonoids Content Measurement: Flavonoids content (FC) was quantified using a colorimetric method [10]. Two hundred and fifty microliters of sample were mixed with 1.25 ml dH_2O and 75 µl of 5% $NaNO_2$ for 6 min. One hundred and fifty microliters of 10% $AlCl_3$ were added and allowed to stand for 5 min and then 0.5 ml of 1M NaOH was added and adjusted to 2.5 ml with dH_2O. The optical absorbance was measured at 510 nm. FC was expressed as micrograms of catechin equivalents (CAE) per milligrams of dried extract.

Free Radical Scavenging Assay: Free radical scavenging activity was determined by DPPH$^{\bullet}$ (1,1-diphenyl-2-picrylhydrazyl) assay [11]. Fifty microliters of sample were mixed with 1.95 ml of DPPH reagent, dissolved in methanol as in instruction, allowed to stand in the dark for 45 min and then measured the absorbance at 515 nm. Ascorbic acid (AA), catechin (CA) and epigallocatechin-3-gallate (EGCG) were served as positive controls. Radical scavenging activity was calculated using the following formula and expressed as median inhibition concentration, IC_{50}.

$$Radical\ scavenging\ activity\ (\%) = \left[1 - \frac{(A_1 - A_2)}{A_0}\right] x\ 100$$

Where:

A_0 : Was the absorbance of a control
A_1 : Was the absorbance of DPPH$^{\bullet}$ solution in the presence of a sample
A_2 : Was the absorbance without DPPH$^{\bullet}$ solution

Ferric Thiocyanate Assay: Ferric thiocyanate (FTC) assay was conducted as described by Huang *et al.* [12]. One milliliter of sample, diluted in 99.5% ethanol, was mixed with 1.5 ml of 2.51% linoleic acid, 2.5 ml of 0.05 M phosphate buffer, pH 7.0 and then kept at 40°C in the dark. To 0.5 ml aliquot of sample, 4.9 ml of 75% ethanol and 50 µl of 30% ammonium thiocyanate were added and incubated for 3 min. Fifty microliters of 20 mM iron (II) chloride in 3.5% hydrochloric acid were added and measured the absorbance at 500 nm every 24 hrs until one day after the absorbance of control reached its maximum. 3-*tert*-butyl-4-hydroxyanisole (BHA), CA and EGCG were used as positive controls. Lipid peroxidation inhibition (LPI) was calculated using the following formula.

$$Lipid\ \ peroxidation\ inhibition\ (\%) = \left[1 - \frac{(A_1 - A_2)}{A_0}\right] x\ 100$$

Where:

A_0 : Was the absorbance of control
A_1 : Was the absorbance in the presence a sample

A_2 : Was the absorbance without potassium thiocyanate solution.

Ctytotoxicity Assay: Cytotoxicity was performed by brine shrimp lethal assay (BSLA) as described by Solis *et al.* [13]. Brine shrimp *Artemia salina* Linn. eggs were purchased from a local fish shop. They were hatched and reared in artificial seawater (120 g/l sea salt) under continuous light, at 25°C for 24 hrs [14]. Ten nauplii were transferred onto a 24-well plate containing 200 µl of artificial seawater and incubated with 800 µl of extract solution at various concentrations for 24 hrs. The dead larvae were counted. The percentage of mortality was calculated as following:

$$Mortality\ (\%) = \left[1 - \frac{(A_1 - A_2)}{A_1}\right] x\,100$$

Where:

A_1 : Was the live control (the medium without the sample)
A_2 : Was the death in the presence of the samples.

The lethal concentrations at 10%, 50% and 90% (LC_{10}, LC_{50} and LC_{90} values) and 95% confidence intervals were determined at 24 h using the Probit analysis method [15] and expressed as micrograms of sample per milliliter. Four repeats were performed.

Toxicological Profile for Pharmacological Development: Concentration-response relationship between LC_{50} of cytotoxicity and IC_{50} of DPPH radical scavenging activity. No-observed-adverse-effect-level (NOAEL), lowest-observed-adverse-effect-level (LOAEL), margin of safety (MOS) and therapeutic index (TI) were analyzed, described by Calabrese [14], Beck [16] and Faustman [17], as the following formula:

$$MOS = \frac{NOAEL}{Effective\ Dose}$$

$$TI = \frac{LC_{50}}{IC_{50}}$$

Cell Proliferation Assay: MCF-7, human breast cancer cell line (a gift from R.P. Shiu, Dubik and Shiu, 1992) was cultured in complete Dubecco's Modified Eagle's medium, DMEM/F-12, supplemented with 10% FBS and 1% penicillin/streptomycin and incubated in 5% CO_2 atmosphere at 37°C. Cell proliferation was assayed by MTT,3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide. MCF-7 cells at 10,000 cells/well in 100 µl were plated onto a 96-well plate and incubated for 24 hrs. The cultured cells were treated with the extracts at various concentrations and continued to incubate for 24 hrs. DMSO was used as control. MTT at 5 µg/µl (in phosphate-buffered saline, pH 7.4) was added and incubated for 4 hrs. The cultured medium was discarded. One hundred and fifty microliters of DMSO were added. The plate was gently agitated until the formazan precipitate was dissolved. The absorbance was measured at 570 nm with the reference wavelength at 630 nm. Decreasing in the absorbance indicated a reduction in cell viability [18]. Four replicates were performed. The antiproliferation activity (%) was plotted against the sample concentrations and the median lethal concentration of 50% (LC_{50}) was derived from the best fit line obtained by linear regression analysis.

$$Antiproliferation\ activity\ (\%) = \left[1 - \frac{(A_1 - A_2)}{(A_0 - A_2)}\right] x\,100$$

Where:

A_0 : Was the absorbance of control
A_1 : Was the absorbance of the treated sample
A_2 : Was the absorbance of treated sample without cells

Statistical Analysis: Data were analyzed for multiple comparisons by one-way ANOVA, using the least significant test to determine the level of significant at $p < 0.05$ and 0.01. For single comparisons, the different significance of means was determined by Student's *t*-test at significant level of $p < 0.05$ and $p \leq 0.01$.

RESULTS

Total Phenolic Compounds and Flavonoids Content: Pomegranate peel ethanolic and water extracts (PPE/e, PPE/w) substantially contained both total phenolic compounds (TPC) and flavonoids content (FC) higher than those of seed extracts (PSE/e, PSE/w) (Table 1). TPCs of all extracts ranged as PPE/e > PPE/w > PSE/e > PSE/w. The TPC amounts of them were 449.60±4.40, 380.54±5.87, 77.93±1.62 and 51.58±0.85 µg GAE/mg respectively. The FC amounts of them were 38.44±1.44, 26.04±0.93, 16.66±0.47 and 10.55±0.14 µg CAE/mg respectively.

Table 1: Total phenolic compounds, flavonoids contents of pomegranate *P. granatum* peel and seed extracts and their antioxidant activities, assessed by DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging and lipid peroxidation inhibition. Data represent mean±SE, n = 4

Sample	Total Phenolics mg GAE/mg	Flavonoids mg GAE/mg	DPPH IC_{50}, µg/ml	LPO Inhibition IC_{50}, µg/ml
PPE/e	449.60±4.40[a]	38.44±1.44[a,a]	121.65±2.66[a]	18.04±1.95[a]
PPE/w	380.54±5.87[a]	26.04±0.93[b,b]	151.78±2.70[a]	22.34±2.11[a]
PSE/e	77.93±1.62[b]	16.66±0.47[c,a]	1,324.35±16.89[b]	166.49±20.38[b]
PSE/w	51.58±0.85[b]	10.55±0.14[d,b]	2,577.53±44.06[c]	201.82±11.37[b]
AA	-	-	113.35±1.95	-
BHA	-	-	-	5.97±0.39
CA	-	-	111.39±0.73	7.38±0.11
EGCG	-	-	65.17±0.34	6.89±0.39

PPE/e, pomegranate peel ethanolic extract; PPE/w, pomegranate peel water extract; PSE/e, pomegranate seed ethanolic extract; PSE/w, pomegranate seed water extract; AA, ascorbic acid; BHA, 3-*tert*-butyl-4-hydroxyanisole; CA, catechin; EGCG, epigallocatechin-3-gallate; IC_{50}, median inhibitory concentration. Numbers with different letters within the same column are significantly different ($P < 0.05$).

Table 2: Cytotoxicity, LC_{10}, LC_{50} and LC_{90}, of pomegranate *P. granatum* peel and seed extracts assayed by brine shrimp lethality (BSLA). Data were mean±SE, n = 4

Extract	Conc. (µg/ml)	Mortality (%)	LC_{10} (µg/ml)	LC_{50} (µg/ml)	LC_{90} (µg/ml)
PPE/e	500	2.50±1.02	777.73±29.60[b]	1,206.98±12.73[a]	1,863.27±40.04[a]
	750	5.00±1.02	(688.53-876.93)	(1,166.46-1,247.50)	(1,735.85-1,990.69)
	1,000	35.63±2.13			
	1,250	52.50±2.28			
	1,500	73.13±2.13			
	1,750	82.50±2.28			
PPE/w	500	1.88±1.20	1,345.93±5.5[d]	1,743.31±20.17[b]	2,115.54±38.03[a]
	750	3.13±0.63	(1,328.41-1,363.46)	(1,679.14-1,807.49)	(1,994.52-2,236.56)
	1,000	3.75±0.72			
	1,250	5.63±1.20			
	1,500	18.13±1.20			
	1,750	51.25±1.61			
	2,000	81.88±4.83			
PSE/e	500	5.00±1.77	1,055.33±104.88[c]	2,375.28±69.54[c]	3,574.37±99.55[c]
	750	5.63±1.57	(721.54-1,389.13)	(2,153.97-2,596.59)	(3,257.54-3,891.20)
	1,000	13.13±4.93			
	1,500	17.50±1.02			
	2,000	38.75±5.15			
	2,500	57.50±2.28			
	3,000	72.50±2.04			
PSE/w	100	1.88±0.63	230.20 ±34.08[a]	1,294.88±61.28[a]	2,416.04±54.09[b]
	500	18.13±3.29	(121.75-338.66)	(1,099.86-1,489.89)	(2,243.89-2,588.20)
	750	33.50±3.34			
	1,000	45.00±5.10			
	1,500	68.75±1.61			
	2,000	76.25±2.60			
	2,500	86.88±2.13			
Control		1.25±0.72			

PPE/e, pomegranate peel ethanolic extract; PPE/w, pomegranate peel water extract; PSE/e, pomegranate seed ethanolic extract; PSE/w, pomegranate seed water extract; Numbers with different letters within the same column are significantly different ($P < 0.01$). Control expressed as 0.001% DMSO

Table 3: Antiproliferative activity of pomegranate *P. granatum* peel and seed extracts on MCF-7 cells. Data were mean±SE, n = 4

Sample	Concentration (µg/ml)	Antiproliferation (%)	LC_{50} (µg/ml)
PPE/e	200	11.48±0.96	375.75±1.22[a]
	300	27.08±1.17	
	400	47.90±2.11	
	500	88.35±0.68	
PPE/w	300	8.47±1.82	471.80±4.37[a]
	400	30.89±1.69	
	500	58.07±2.74	
	600	81.52±1.16	
PSE/e	1,600	7.26±0.65	1,786.58±6.74[b,a]
	1,700	30.07±2.49	
	1,800	50.64±3.87	
	1,900	80.74±1.54	
PSE/w	5,000	23.88±4.73	7,969.16±143.37[c,b]
	6,000	34.68±2.78	
	7,000	45.03±1.83	
	8,000	51.44±0.53	
EGCG	200	60.18±0.49	179.23±1.22

PPE/e, pomegranate peel ethanolic extract; PPE/w, pomegranate peel water extract; PSE/e, pomegranate seed ethanolic extract; PSE/w, pomegranate seed water extract; EGCG, epigallocatechin-3-gallate. Numbers with different letters within the same column are significantly different ($P < 0.01$)

Table 4: Toxicological profiles of pomegranate *P. granatum* peel and seed extracts were statistically derived from cytotoxicity and antioxidant activities

			DPPH		LPI	
Sample	NOAEL (µg/ml)	LOAEL (µg/ml)	MOS	TI	MOS	TI
PPE/e	750	1,000	150	9.92	150	66.91
PPE/w	1,250	1,500	250	11.49	250	78.04
PSE/e	1,000	1,500	10	1.79	20	14.27
PSE/w	100	500	1	0.50	2	6.42

PPE/e, pomegranate peel ethanolic extract; PPE/w, pomegranate peel water extract; PSE/e, pomegranate seed ethanolic extract; PSE/w, pomegranate seed water extract; NOAEL, no observed adverse effect level; LOAEL, lowest observed adverse effect level; MOS, margin of safety; TI, therapeutic index

Free Radical Scavenging Activity: The pomegranate peel extracts exhibited proton-donating ability by DPPH assay. PPE/e demonstrated highest free radical scavenging activity with IC_{50} value of 121.65±2.66 µg/ml, followed by PPE/w, PSE/e and PSE/w with IC_{50} of 151.78±2.70, 1,324.35±16.89 and 2,577.53±44.06 µg/ml, respectively (Table 1). The activity of PPE/e was prominent and similar to those of AA and CA the antioxidant standards, but two fold less than that of EGCG. The extract scavenging activity, IC_{50}, was well correlated to total phenolic compounds, $R^2 = 0.833$, $P < 0.05$ (Figure 1 A) and to flavonoids content, $R^2 = 0.792$, $P < 0.05$ (Figure 1 B).

Lipid Peroxidation Inhibition: PPE/e possessed highest inhibitory activity on lipid peroxidation (LPO) of linoleic acid with IC_{50} value of 18.04±1.95 µg/ml (Table 1). Pomegranate peel extracts, however, moderately inhibited LPO as compared to the standards. This activity was approximately 2.5-3 fold less than those of BHA, CA and EGCG. PPE/w contained moderate LPO inhibition with IC_{50} of 22.34±2.11 µg/ml. The LPO inhibitory activities of PSE/e and PSE/w were 166.49±20.38 and 201.82±11.37 µg/ml, respectively, which were about 25-30 fold less than those of the standard activities. The correlation between IC_{50} values of lipid peroxidation inhibition and TPC was high with $R^2 = 0.976$, $P < 0.05$ (Figure 1 C) and FC was moderate with $R^2 = 0.835$, $P < 0.05$ (Figure 1 D).

Cytotoxic Effect: The cytotoxicity of the pomegranate extracts as performed by BSLA was low. The LC_{10}, LC_{50} and LC_{90} values at 24 hrs of the extracts were shown in Table 2. PSE/w seemed to be most toxic at LC_{10}, while PPE/e showed the highest at LC_{50} and LC_{90}. At LC_{10}, the cytotoxicity ranged as PSE/w > PPE/e > PSE/e > PPE/w with LC_{10} values of 230.20±34.08, 777.73±29.60, 1,055.33±104.88 and 1,345.93±5.51 µg/ml. At LC_{50}, it ranged as PPE/e > PSE/w > PPE/w > PSE/e with the values of 1,206.98±12.73, 1,294.88±61.28, 1,743.31±20.17 and 2,375.28±69.54 µg/ml, respectively. At LC90, it ranged as PPE/e > PPE/w > PSE/w > PSE/e with the values of

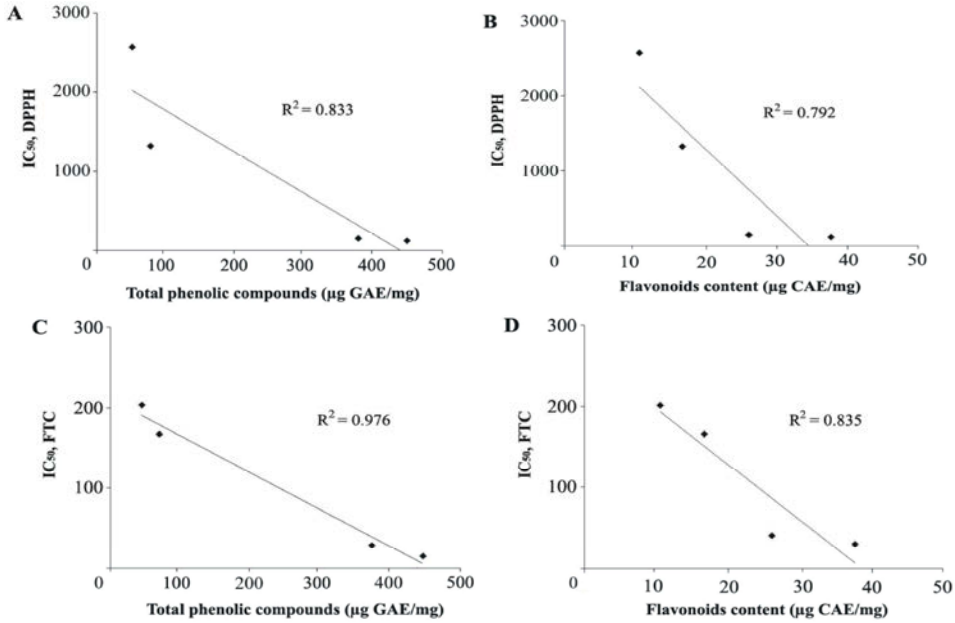

Fig. 1: Relationships between IC_{50} of free radical scavenging activity, by DPPH method, and total phenolic compounds (A), and flavonoids content (B) of P. granatum peel and seed extracts. Relationships between IC_{50} of lipid peroxidative inhibition, by FTC method, and total phenolic compounds (C), and flavonoids content (D) of P. granatum peel and seed extracts

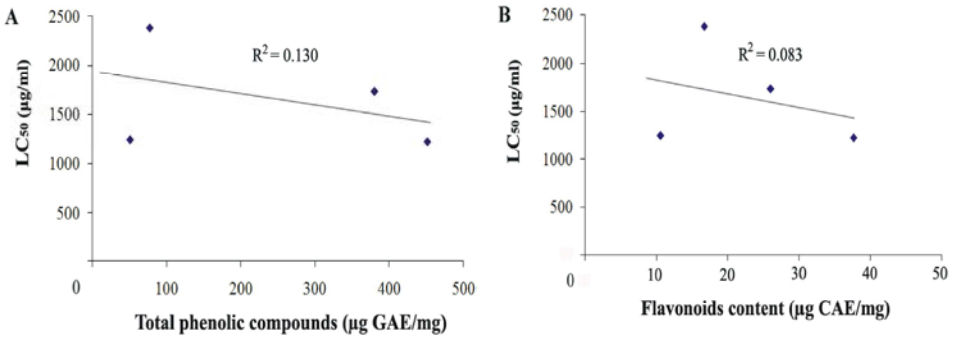

Fig 2: Relationships between LC_{50} of motality and total phenolic compounds (A), and flavonoids content (B) of pomegranate P. granatum peel and seed extracts

1,863.27±40.04, 2,115.54±38.03, 2,416.04±54.09 µg/ml, respectively. However, the cytotoxicity of the pomegranate extracts was not correlated to their TPC and FC contents (Figure 2).

Antiproliferation Effect on MCF-7 Cells: The cytotoxicity of the pomegranate extracts was also evaluated by their activities on the proliferation of a human breast adenocarcinoma cell line, MCF-7 cells. Interestingly, pomegranate peel extracts were prominently potent against the proliferation of MCF-7 cells. The antiproliferation potency, LC_{50} at 24 h, of PPE/e and PPE/w was 375.75±1.22 and 471.80±4.37 µg/ml (Table 3). The effect of PSE/e was moderate with LC_{50} of 1,786.58±6.74 µg/ml while PSE/w was least effective with LC_{50} of 7,969.16±143.37 µg/ml.

Concentration-Response Functions for Pomegranate Extracts: The NOAEL, LOAEL, MOS and TI were identified based on the statistic significance of PPE cytotoxicity by BSLA. The NOAEL values of the pomegranate extracts ranged as PPE/w > PSE/e > PPE/e > PSE/w with concentrations of 1,250, 1,000, 750 and 100 µg/ml respectively (Table 4). While, the LOAEL values ranged as PPE/w = PSE/e > PPE/e > PSE/w with concentrations of 1,500, 1,000 and 500 µg/ml respectively.

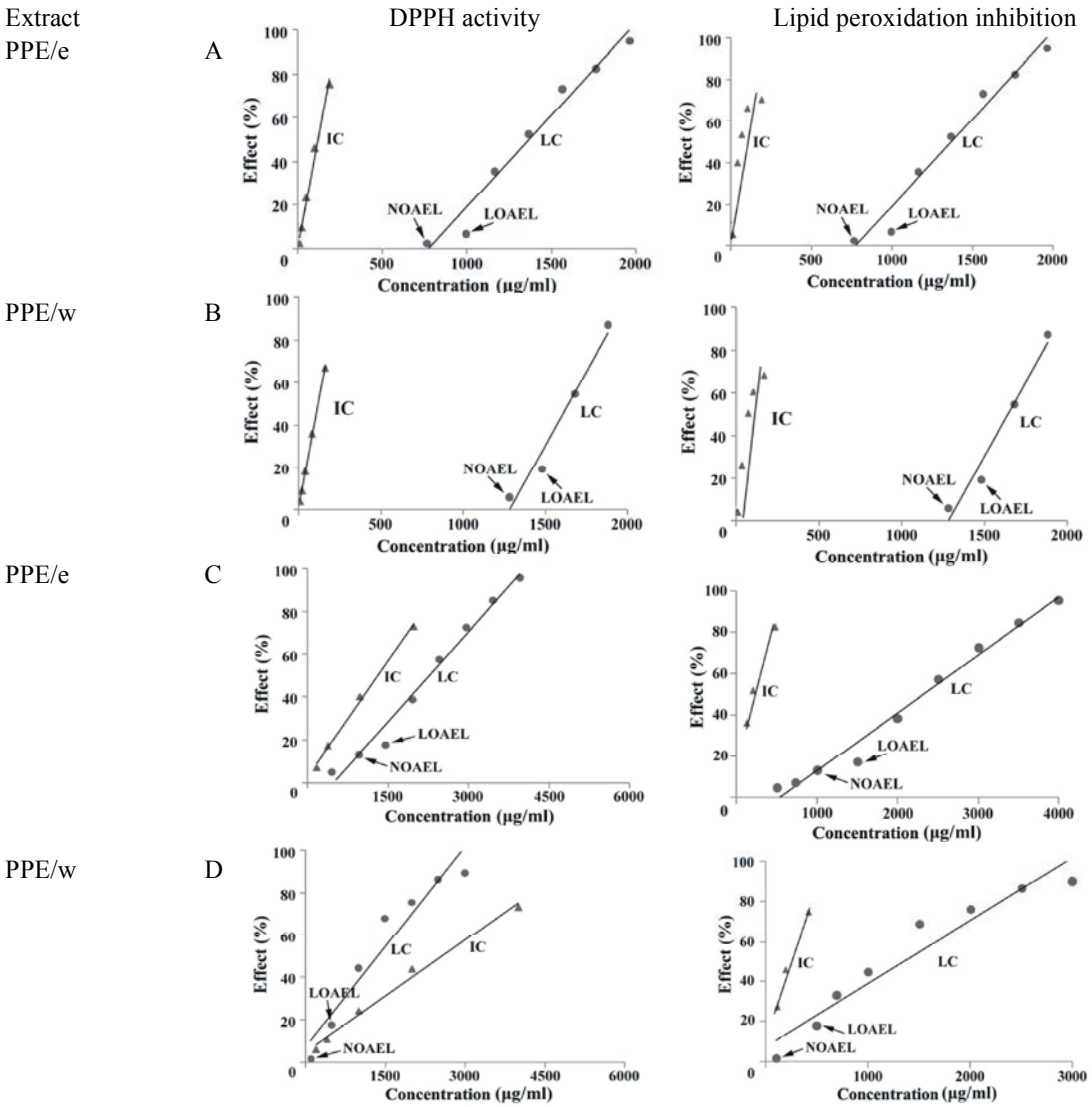

Fig 3: In vitro concentration-response relationships of LC_{50} of cytotoxicity evaluated by brine shrimp lethality assay and IC_{50} of DPPH radical scavenging activity (Lt. panels) and IC50 of lipid peroxidation inhibition and (Rt. panels).

The concentration-response relationships between IC_{50} values of antioxidant activities and LC_{50} values of cytotoxicities and effects of the extracts were showed in Figure 3.

The MOS and the TI of the extract antioxidant effects by DPPH and LPI were as similar pattern to NOAEL and LOAEL, i.e., MOS and TI values were PPE/w > PSE/e > PPE/e > PSE/w (Table 3). PSE/w possessed lowest MOS of 1 and 2; and TI of 0.50 and 6.42, based on DPPH radical scavenging (Figure 3, Lt. panels) and LPI (Figure 3, Rt. panels) respectively. PPE/w showed highest MOS values of 250; and TI of 11.49 and 78.04 respectively.

DISCUSSION

Phytochemical constituents of plants importantly indicate the antioxidant capacity and cytotoxicity of their products which can be used for human health and drug development.

Pomegranate *Punica granatum* fruits were a rich source of dietary antioxidants [19]. We found that different parts of pomegranate fruits and extracting solvents produced different quantities of different phytochemicals leading to different magnitude of biological activity of the products. PPE possessed higher phenolics and flavonoids than PSE and the ethanolic

extracts of them contained more phenolics than flavonoids [6, 20]. There were some reports that PPE was rich in gallic acid, ellagic acid, flavonols, flavones, flavanones, anthocyanidins and ellagitannins [21-23]. While, PSE was a source of fatty acids, i.e. punicic acid, linoleic acid, oleic acid, palmitic acid and stearic acids [24] and non-steroid phytochemicals [25]. These reports thus well agreed with our findings.

The substantial amounts of TPC and FC in PPEs were well correlated and dominantly responsible for the antioxidant activity. We found that PSEs possessed less antioxidant activity as other reports [26]. PPE potently scavenged DPPH radicals similar to catechin, it is likely that PPE possessed proton-donating ability and in association with a number of hydroxyl groups in the TPC and FC structures to stabilize free radicals [20, 27-30]. The pomegranate extracts prominently inhibited lipid peroxidation, it could due to their TPC and FC ability to quench hydroxyl radicals by transferring hydrogen atom to free radical [31]. The inhibition of lipid peroxidation activity of the by-product pomegranate peel is one of the important roles of antioxidants which protect biological membranes of living cells, leading to attenuation atherosclerosis [23], hyperlipidemia and diabetic [32]. The antioxidant capacity of PPE was also valuable for increasing shelf life of lipid containing food [33, 34] and meat products [35, 36]. PPE was demonstrated that it well protected the UVB-irradiated rat skin by remarkably reducing skin lesion and DNA fragmentation of the epidermal cells [37].

We found that PPE and PSE of this study did not show cytotoxicity to normal living organism (brine shrimps), i.e. LC_{50} exceeded 1,000 µg/ml [38, 39], which supported by other reports [28, 40, 41]. However, PPE inhibited the proliferation of MCF-7 cells, a human breast adenocarcinoma cell line. The antiproliferation of other cancer cells by PPE was reported, such as HL-60, human promyelocytic leukemia cells [42], MCF-7aro, testosterone-induced breast cancer cells [43], LNCaP-AR and DU-145 cells, prostate cancer cells [44] and PANC-1 cell, pancreatic cancer cells [45]. The cytotoxic and antioxidant studies of pomegranate extracts were useful for prediction of their risk. Similarly, the NOAEL of resveratrol [46], neem-derived pesticides [47], *Tancetum vulgare* [48] and *Herniaria glabra* [49] leaf aqueous extracts, vitamin D [50], copper in bottled drinking water [51] and some environmental chemicals [52] was evaluated for risk assessment and safe consumption. The statistically predicted values of NOAEL, LOAEL, MOS and TI of the pomegranate peel and seed extracts were firstly evaluated for acute toxicity by our study. It was likely indicated that the pomegranate peel and seed extracts were highly effective with low toxicity for normal cell treatments and preferably accepted as potent antioxidants. Thus, these concentration-response relationships provide the important data for the toxicological risk assessment and the implications for pharmacological development of pomegranate products.

CONCLUSION

Pomegranate peel and seed extracts were rich in phenolic and flavonoid compounds with potentially high antioxidant activities, less cytotoxicity to normal cells, high antiproliferation against cancerous MCF-7 cells. The peel and seed extracts were low cytotoxicity with high safety. Particularly, the peel, the by-product of pomegranate fruits was prominently value added. The toxicological risk assessment and pharmacological data from this pomegranate study would be beneficial for clinically therapeutic development.

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