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# Comparison of antioxidant capacities and cytotoxicities of certain fruit peels

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**Abstract**

This work was undertaken to explore the potential of fruit waste materials as sources of powerful natural antioxidants. The peels of eight kinds of fruits commonly consumed and grown in Thailand were used. The ethanolic fruit peel extracts were subjected to the scavenging tests of DPPH and ABTS radicals. Results from both assays were in good agreement that the top three markedly high free radical-scavenging power was from the peel extracts of *Punica granatum* (pomegranate), *Nephelium lappaceum* (rambutan), and *Garcinia mangostana* (mangosteen). The $IC_{50}$ values to quench the DPPH free radicals of these three extracts were 0.003, 0.006, and 0.023 mg/ml and the trolox equivalent antioxidant capacity (TEAC) values from ABTS assay were 4.066, 3.074, and 3.001 mM/mg, respectively. The extract of mangosteen peel showed moderate toxicity to Caco-2 cells and high toxicity to peripheral blood mononuclear cells (PBMC) with the $IC_{50}$ values of 32.0 and 4.9 μg/ml, respectively. Pomegranate peel extract stimulated Caco-2 cell and PBMC proliferation with the $ED_{50}$ of 4.7 and 44.4 μg/ml, respectively. Peel extract of rambutan exhibited extremely high value of $IC_{50}$ (>100 μg/ml) against both cell types indicating non-toxic activity to the cells. It was concluded that the peel of rambutan may be considered potentially useful as a source of natural antioxidants for food or drug product because of its high antioxidant activity and non-toxic property to normal cells.


*Keywords:* Pomegranate; Rambutan; Mangosteen; Antioxidant; Cytotoxicity; ABTS; DPPH; Caco-2; PBMC

## 1. Introduction

In the past, many synthetic antioxidants have been popularly used in food and pharmaceutical formulations. Recently, the demand for natural antioxidants has increased, due to consumer concerns about the safety of synthetic antioxidants (Hudson, 1990; Shahidi, 1997). Among various plants, fruit is important as a natural antioxidant source. The antioxidant activity in fruits is notable since fruits are rich in compounds that have an important role in free radical-scavenging activity. Those compounds are polyphenols, such as flavonoids, tannins, and catechins (Macheix, Fleuriet, & Billot, 1990). Moreover, fruits contain many vitamins, which express antioxidant activity, e.g. vitamin C, vitamin E, and β-carotene (Paul & Southgate, 1978; Hernandez, Lobo, & Gonzalez, 2006). The antioxidant activity in fruits varies among species and cultivars (Award, de Jager, van der Plas, & van der Krol, 2001; Kondo, Jitratham, Kittikorn, & Kanlayanarat, 2004). The diverse antioxidant compounds present in fruits are responsible for the high antioxidant capacity. Many experiments have reported the antioxidant activity of fruit juice and fruit pulp from edible fruits (Mokbel & Hashinaga, 2006; Valcheva-Kuzmanova, Borisova, Galunska, Krasnaliev, & Belcheva, 2004; Bub et al., 2003). However, there is a little information on the antioxidant activity in fruit peels.

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Peels are often the waste part of various fruits. These wastes have not generally received much attention with a view to being used or recycled rather than discharged. This might be due to their lack of commercial application (Soong & Barlow, 2004). Interestingly, the peel and seed fractions of some fruits have higher antioxidant activity than the pulp fractions (Jayaprakasha, Sigh, & Sakariah, 2001). For example, pomegranate peel has a higher antioxidant activity than its pulp (Li et al., 2006). Apple fruit peel was reported to be acclimatised to the prevailing light exposure within the tree canopy to meet the respective needs for dissipating excess absorbed photon flux density and detoxifying reactive oxygen free radical species (Ma & Cheng, 2003). Grape seed is higher than its pulp in antioxidant capacity and is a rich source of proanthocyanidin, which is very effective in scavenging various reactive oxygen free radical species (Guo et al., 2003).

Thailand has a diversity of edible fruits. However, only some of them are popularly consumed. Due to the high consumption of their edible parts, their peels (as waste products) have been discharged, causing a severe problem in the community as they gradually ferment and release off odours. As part of our ongoing research on antioxidants from natural resources, the peels of popular fruits, namely mangosteen, rambutan, banana, pomegranate, coconut, long-gong, passion fruit and dragon fruit, were selected for study. All are grown in Thailand. The edible parts of some of these fruits, e.g. pomegranate and mangosteen, have been reported to have high antioxidant activity (Gil, Tomas-Barberan, Hess-Pierce, Holcroft, & Kader, 2000; Schubert, Lansky, & Neeman, 1999; Williams, Ongsakul, Proudfoot, Croft, & Bellin, 1995). However, research on the antioxidant activity of the peels from such popular fruits has been scarce. Moreover, the cytotoxicity, particularly to normal cells of those peels, has not yet been reported. The aim of this study was to investigate and compare the antioxidant activity and cytotoxicity of fruit peels commonly consumed and grown in Thailand in order to search for a powerful non-toxic antioxidant source among them.

## 2. Materials and methods

### 2.1. Materials

#### 2.1.1. Fruits

Eight species of fruits most commonly consumed and grown in Thailand were purchased from a local grocery store. They were coconut (*Cocos nucifera*), mangosteen (*Garcinia mangostana*), dragon fruit (*Hylocereus undatus*), long-gong (*Lansium domesticum*), banana (*Musa sapientum*), rambutan (*Nephelium lappaceum*), passion fruit (*Passiflora foetida*) and pomegranate (*Punica granatum*).

#### 2.1.2. Chemicals

Trolox, potassium persulfate, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), dimethyl sulfoxide (DMSO), 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyl tetrazolium bromide (MTT), non-essential amino acid (NEAA), and Histopaque®-1077 were purchased from Sigma–Aldrich (St. Louis, MO, USA). Dulbecco's modified eagle medium (DMEM), RPMI 1640, trypsin–EDTA, and penicillin–streptomycin were from GIBCO™ Invitrogen (Grand Island, NY, USA). Fetal bovine serum (FBS) was obtained from Biochrom AG (Berlin, Germany) and ethanol from Fluka Chemicals (Buchs, Switzerland). All other chemicals were of the highest grade available.

### 2.2. Sample preparation

The dried fruit peels were ground into fine powder and extracted with 95% ethanol for three days, thrice, at room temperature. The filtrates were pooled and concentrated by rotary evaporator at 45 °C. The obtained semisolid extracts were kept in a desiccator at 4 °C until further used.

### 2.3. Antioxidant activity

#### 2.3.1. DPPH method

The scavenging activity on DPPH radical of all fruit peel extracts was determined by modifying the methods of Gamez et al. (1998) and Brand-Williams et al. (1995). The extracts were mixed with ethanol to prepare an ethanolic test solution of different concentrations (0.1–1.0 mg/ml). DPPH was dissolved in ethanol and mixed with a certain amount of the ethanolic test solution. The solution was adjusted to a final DPPH concentration of 100 μM. The mixture was shaken vigorously and left to stand for 30 min in the dark at room temperature. The amount of DPPH remaining in each period of stand was determined spectrophotometrically at 540 nm using a microtitre plate reader. All measurement was performed in triplicate. The radical-scavenging activity was calculated as % inhibition from the following equation:

$$\%\ \text{Inhibition} = \frac{\text{OD}_{\text{blank}} - \text{OD}_{\text{sample}}}{\text{OD}_{\text{blank}}} \times 100$$

#### 2.3.2. ABTS method

The pre-formed radical monocation of 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\cdot+}$) was generated according to the modified method of Re et al. (1999) by oxidation of ABTS solution (7 mM) with 2.45 mM potassium persulfate ($K_2S_2O_8$). The mixture was allowed to stand for 12 h in the dark at room temperature. The ABTS solution was diluted with ethanol to the absorbance of $0.7 \pm 0.2$ at 750 nm. Series of extract solutions were prepared by dissolving in ethanol to yield a concentration of 1 mg/ml. An aliquot of 20 μl of each ethanolic solution was added to 180 μl of $ABTS^{\cdot+}$ radical cation solution. The absorbance was monitored spectrophotometrically at 750 nm for 60 min by using a microtitre plate reader. All measurements were performed in triplicate. The antioxi-

dant activity of each sample was expressed as trolox equivalent antioxidant capacity (TEAC), which obtained by comparing the absorbance change at 750 nm in a reaction mixture containing a sample of plant extract with that containing trolox. This index is defined as the millimolar concentration of a trolox solution whose antioxidant capacity is equivalent to 1.0 mg of the extract (Antolovich, Prenzler, Patsalides, McDonald, & Robards, 2002).

### 2.4. Cytotoxicity

#### 2.4.1. Caco-2 cell culture

The Caco-2 cells (ATCC, Manassas, VA) were routinely cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum (FBS), 1% non-essential amino acids, 100 U/ml of penicillin, and 0.1 mg/ml of streptomycin in a humidified atmosphere of 5% $CO_2$ at 37 °C. The cells were used from passage 64–75. The culture medium was renewed on alternate days. When they had reached confluence, the cells were passaged at a split ratio of one to four by trypsinizing with 0.1% trypsin and 0.02% EDTA in phosphate-buffered saline (PBS). The cytotoxicity study was performed when the cells grew up to 80–90% confluency and were seeded into the 96-well plates at a density of $8 \times 10^3$ cells/well.

#### 2.4.2. Preparation of peripheral blood mononuclear cells (PBMC)

Blood samples from healthy volunteers were collected by venipuncture and transferred into 15 ml of heparin-coated test tubes. Blood was diluted at 1:1 ratio with PBS and layered onto Histopaque®-1077 at a volume ratio of 3:1 and centrifuged at 1000*g* for 30 min. During centrifugation, the PBMCs move from the plasma and are suspended in the density gradient isolating them from erythrocytes and granulocytes. The PBMC layer was removed and then washed twice with PBS. The supernatant was then removed and the cells were resuspended in RPMI1640 culture medium supplemented with 10% FBS, 100 U/ml of penicillin, 0.1 mg/ml of streptomycin and an appropriate amount of sodium bicarbonate. Cell viability was determined by using the trypan-blue dye exclusion method. The cell density of PBMC used in the cytotoxicity study was $1 \times 10^5$ cells/well of the 96-well tissue culture plate.

#### 2.4.3. Measurement of cytotoxicity activity

A standard MTT assay (Alley, Scudiero, & Monks, 1988) was performed by plating Caco-2 cells or PBMCs in 96 well plates, in 100 μl of medium per well, and incubating before crude plant extract treatment at 37 °C for 48 h for Caco-2 and 24 h for PBMCs. After the incubation, various concentrations of crude plant extracts (5–100 μg/ml) in a medium (100 μl) were added to the cells and incubated for another 48 h. Each extracted concentration was added into three separated wells. The metabolic activity in each well was determined by the MTT assay and compared with untreated cells. Briefly, after removal of 100 μl of medium, MTT stock dye solution (5 mg/ml MTT dye in PBS) was added (15 μl/100 μl medium) to each well, and the plate was incubated at 37 °C in 5% $CO_2$ atmosphere. After 4 h, supernatant was removed, followed by an addition of DMSO (200 μl) to each well and mixed thoroughly to dissolve the dye crystals. The absorbance was measured by using an ELISA plate reader at 570 nm with a reference wavelength of 630 nm. The experiment was done in triplicate. The cell viability was determined by the following formula:

$$\%\ \text{Cell viability} = \frac{\text{Mean absorbance in test wells}}{\text{Mean absorbance in control wells}} \times 100$$

Dose–response curves between percentages of cell viability and concentrations of the extracts were constructed. The 50% inhibitory concentration ($IC_{50}$), in the case of cell inhibition, or effective concentration ($ED_{50}$), in the case of cell stimulation, was determined from the plotted curve.

## 3. Results and discussion

### 3.1. The yield of extracts

Table 1 shows the percent yield of tested fruit peel extracts. The highest (10.68%) and lowest (1.40%) yields of extraction were obtained from the peels of rambutan and dragon fruit, respectively.

### 3.2. Radical-scavenging activity on DPPH

The radical-scavenging activity on DPPH was expressed as $IC_{50}$. This value was the concentration of the extract required to inhibit 50% of the initial DPPH free radical. The $IC_{50}$ of all peel extracts is shown in Table 2. Peel extracts of different kinds of fruits possessed obviously different antioxidant activities. The $IC_{50}$ values ranged from 0.003 to 1.291 mg/ml. The extract of pomegranate peel showed the highest antioxidant activity, with an $IC_{50}$ of 0.003 mg/ml, followed by the peel extracts of rambutan and mangosteen, with $IC_{50}$ values of 0.006 and 0.023 mg/ml, respectively. The weakest antioxidant activity was obtained from the extract of long-gong peel with an $IC_{50}$ of 1.29 mg/ml.

Table 1
Percent yield of extracts from different kinds of fruit peels

| Plant name | | % Yield |
|---|---|---|
| Common name | Scientific name | |
| Rambutan | *Nephelium lappaceum* | 10.68 |
| Banana | *Musa sapientum* | 7.66 |
| Mangosteen | *Garcinia mangostana* | 7.21 |
| Pomegranate | *Punica granatum* | 6.21 |
| Coconut | *Cocos nucifera* | 5.80 |
| Long-gong | *Lansium domesticum* | 5.71 |
| Passion fruit | *Passiflora foetida* | 2.60 |
| Dragon fruit | *Hylocereus undatus* | 1.40 |

Table 2
$IC_{50}$ values of fruit peel extracts by DPPH radical method

| Plant name | | $IC_{50}$ (mg/ml)[a] |
|---|---|---|
| Common name | Scientific name | |
| Pomegranate | *Punica granatum* | 0.003 ± 0.002 |
| Rambutan | *Nephelium lappaceum* | 0.006 ± 0.003 |
| Mangosteen | *Garcinia mangostana* | 0.023 ± 0.007 |
| Banana | *Musa sapientum* | 0.031 ± 0.004 |
| Coconut | *Cocos nucifera* | 0.047 ± 0.005 |
| Dragon fruit | *Hylocereus undatus* | 0.084 ± 0.016 |
| Passion fruit | *Passiflora foetida* | 0.104 ± 0.014 |
| Long-gong | *Lansium domesticum* | 1.29 ± 0.001 |

[a] Mean ± SD ($n = 3$).

### 3.3. Radical-scavenging activity on ABTS

The ABTS free radical assay can be used to measure the antioxidant activity of a broad diversity of substances, e.g., both aqueous phase radicals and lipid peroxyl radicals (Rice-Evans, Miller, & Paganga, 1996; Robert et al., 1999). In this experiment, the ABTS method was used to confirm the results from the DPPH test since it is based on a similar antioxidant mechanism and the extracts used in both tests were ethanol-soluble. The scavenging activity of the extracts on free radical ABTS, generated by potassium persulfate was compared with a standard amount of trolox. The result, calculated as trolox equivalent antioxidant capacity (TEAC), is shown in Table 3. All fruit peel extracts possessed the free radical-scavenging property but in different degrees. The TEAC values ranged widely from 0.207 to 4.07 mM/mg. The extract of pomegranate peel exhibited the highest TEAC value of 4.07 mM/mg, followed by that of rambutan and mangosteen with TEAC values of 3.07 and 3.00 mM/mg. The lowest TEAC value, which indicated the weakest antioxidant activity, was obtained from long-gong peel extract. This was in good agreement with that of the DPPH assay. The relationship between TEAC values and $IC_{50}$ of the samples was non-linear, as shown in Fig. 1. However, the plot of logarithmic values of $IC_{50}$ against TEAC gave good linearity with $R^2 = 0.9167$, as shown in Fig. 2, indicating similar trends in the free radical- and hydroxyl radical-scavenging activities.

Regarding these results, it could be considered that the peel extracts of pomegranate, mangosteen, and rambutan contained strong antioxidative agent(s), and pomegranate had the highest potential. However, the utilization of these extracts as antioxidative resources should not be introduced unless their safety has been sufficiently proven. Pomegranate fruit was reported to have high antioxidant activity (Gil et al., 2000; Schubert et al., 1999). The peel of this fruit had recently been reported to have higher antioxidant activity than its pulp and seed (Li et al., 2006). However, Ferrara, Schettino, Forgione, Rullo, and Di Gennaro (1989) reported that some galenic preparations of pomegranate were toxic because of their alkaloid contents. There were some reports of immunological disturbance after consumption of pomegranate fruit (Igea et al., 1991; Gaig et al., 1992). Vidal et al. (2003) revealed possible toxic effects of *P. granatum* extracts in view of their anti-influenza activity. It was shown that toxic effects of *P. granatum* fruit extract occurred at higher doses than those effective in the models where its anti-viral activity had been studied. Therefore, study of cytotoxicity, particularly against normal cells, should be considered as a need before the active agent from these extracts can be generally utilized.

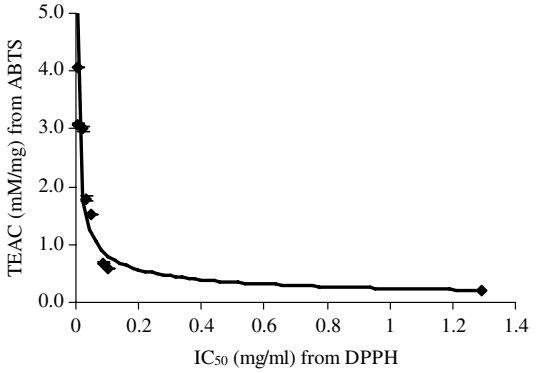


Fig. 1. Correlation of antioxidant activity of fruit peel extract from DPPH ($IC_{50}$) and ABTS (TEAC) assay.

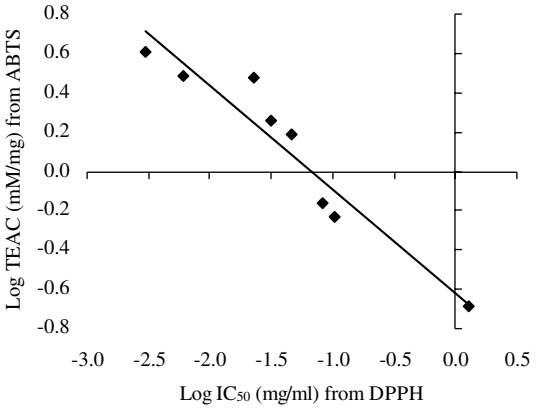


Fig. 2. The linear relationship of logarithmic values of $IC_{50}$ and TEAC.

Table 3
TEAC values of fruit peel extracts

| Plant name | | TEAC (mM/mg)[a] |
|---|---|---|
| Common name | Scientific name | |
| Pomegranate | *Punica granatum* | 4.59 ± 0.009 |
| Rambutan | *Nephelium lappaceum* | 3.07 ± 0.003 |
| Mangosteen | *Garcinia mangostana* | 3.00 ± 0.016 |
| Banana | *Musa sapientum* | 1.80 ± 0.038 |
| Coconut | *Cocos nucifera* | 1.53 ± 0.044 |
| Dragon fruit | *Hylocereus undatus* | 0.685 ± 0.001 |
| Passion fruit | *Passiflora foetida* | 0.591 ± 0.008 |
| Long-gong | *Lansium domesticum* | 0.207 ± 0.002 |

[a] Mean ± SD ($n = 3$).

### 3.4. The cytotoxicity test

The Caco-2 cell line and PBMC were used for the cytotoxicity test in this study. The Caco-2 cell line is derived

from a human colon adenoma and has been used routinely in drug absorption screening, because the Caco-2 monolayer displays several features of the small intestinal epithelial barrier (Hillgren, Kato, & Borchardt, 1995). The cytotoxicity against the Caco-2 cell line could provide preliminary information for study of toxicity on the normal intestinal cell type and for the selection of appropriate concentrations required in a future permeability study of active components. The potential toxicity of the extract on normal cells was assessed by the cytotoxicity test against human PBMC. Many studies had utilized the PBMC to assess the effects of chemicals or extracts on the proliferation of normal cells (Anazetti, Melo, Duran, & Haun, 2003; Liu et al., 2004). Figs. 3 and 4 demonstrate the dose–response curve of the fruit peel extracts against Caco-2 cell line and PBMC, respectively. From these curves, inhibition or stimulation of the cells could be observed. The $IC_{50}$ was obtained when the activity was inhibition whereas the $ED_{50}$ was obtained when the activity was stimulation. The cytotoxicity against Caco-2 cell line and human PBMC of all tested fruit peel extracts is summarized and shown in Table 4. The results show that most

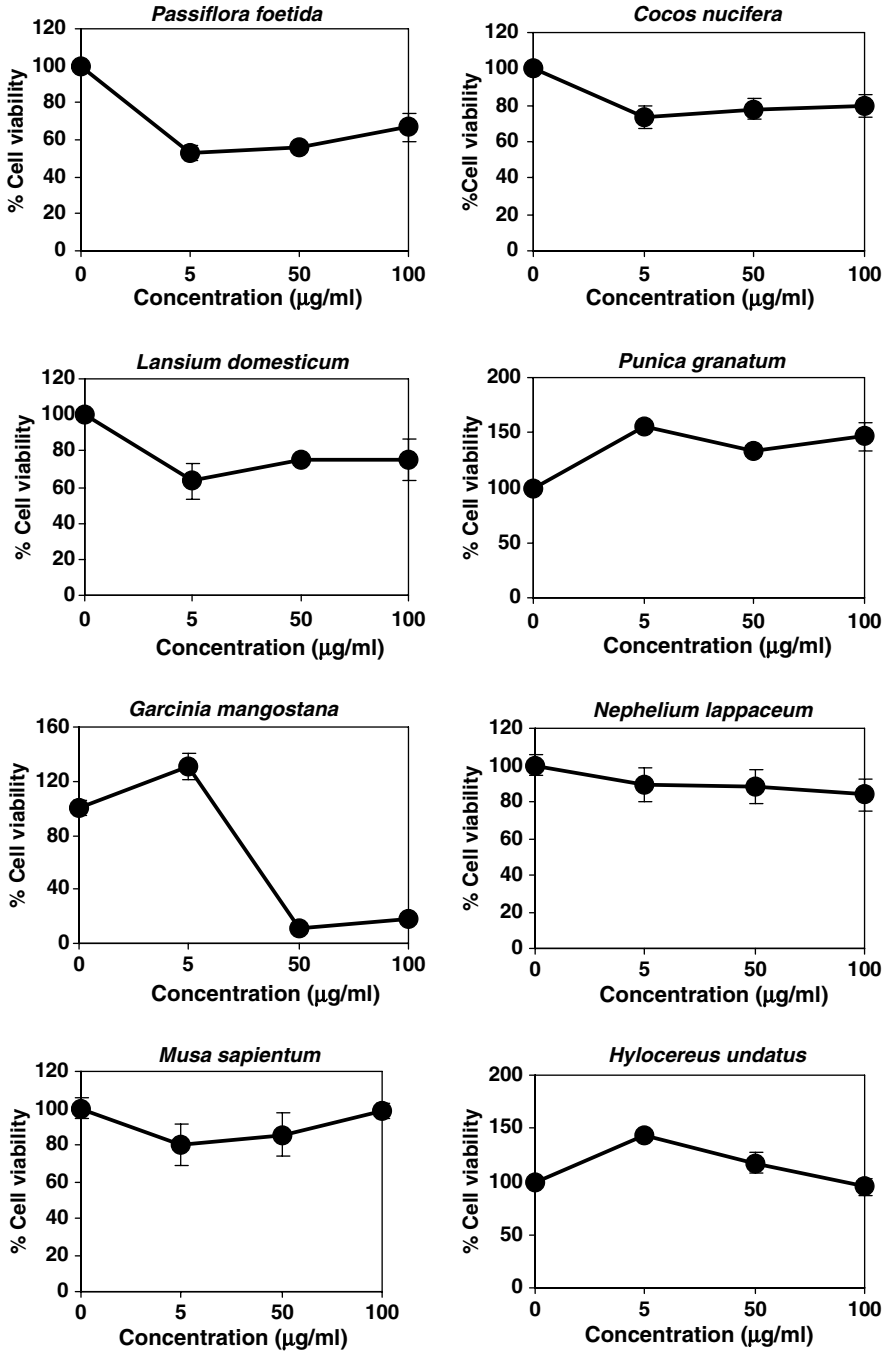


Fig. 3. Effect of the extracts on Caco-2 cells. Cells were grown in the presence of various concentrations of the plant extracts. The number of viable cells was determined by MTT assay in triplicate.

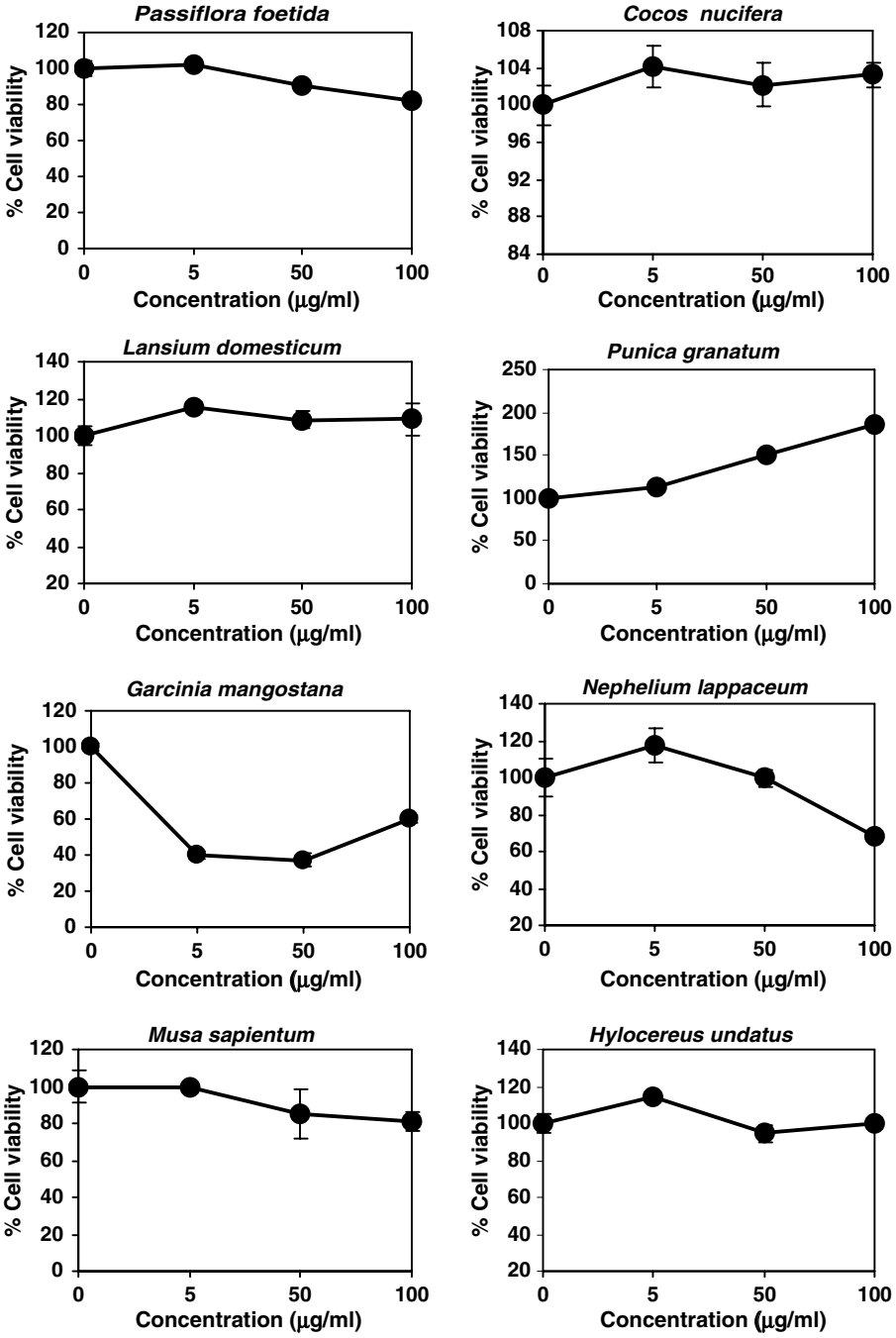


Fig. 4. Effect of the extracts on PBMC. Cells were grown in the presence of various concentrations of the plant extracts. The number of viable cells was determined by MTT assay in triplicate.

of the fruit peel extracts included in this study had no cytotoxic activity against either cell type, except those from mangosteen and pomegranate peels. This mangosteen peel extract exhibited potential toxicity against Caco-2 cells and PBMC with the $IC_{50}$ values of 32.0 and 4.9 μg/ml, respectively. This indicated that the extract of mangosteen peel contained potential cytotoxic agent(s). Therefore, further purification to eliminate the toxic agent(s) might be beneficial for products containing the extract of the mangosteen peel. The extract of pomegranate peel showed stimulation of cell proliferation in both Caco-2 cells and PBMC with the $ED_{50}$ values of 4.7 and 44.4 μg/ml, respectively. This suggested that the application of this extract, as a natural antioxidant in a food or drug for humans, should be treated with caution. Among the three extracts which possessed high antioxidative activity, rambutan peel extract exhibited the highest value of $IC_{50}$ (>100 μg/ml) against both cell types, indicating its mild activity to the cells. According to the standard National Cancer Institute criteria (Chen et al., 1988; Geran, Greenberg, Macdonald, Schumacher,

Table 4
Cytotoxic activities of the studied extracts against Caco-2 cell line and PBMC

| Plant name | Cell Types | |
|---|---|---|
| | Caco-2 | PBMC |
| *Punica granatum* | Stimulate[a] | Stimulate[b] |
| *Nephelium lappaceum* | Inactive | Inactive |
| *Garcinia mangostana* | Inhibit[c] | Inhibit[d] |
| *Musa sapientum* | Inactive | Inactive |
| *Cocos nucifera* | Inactive | Inactive |
| *Hylocereus undatus* | Inactive | Inactive |
| *Passiflora foetida* | Inactive | Inactive |
| *Lansium domesticum* | Inactive | Inactive |

[a] $ED_{50} = 4.7$ μg/ml.
[b] $ED_{50} = 44.4$ μg/ml.
[c] $IC_{50} = 32.0$ μg/ml.
[d] $IC_{50} = 4.9$ μg/ml.

& Abbott, 1972), rambutan peel extract could be classified as non-toxic to normal human cells. It could be used as a natural source of antioxidant or even as a cancer-preventive agent since antioxidative agents are usually found to possess cancer-prevention activity. A good cancer-preventive agent should show inhibitory activity toward cancer cell growth, but not cause serious damage to human PBMC. Anazetti et al. (2003) studied dehydrochrotonin (DHC) which is a diterpene lactone obtained from *Croton cajucara* (Sacaca) and dimethylamide-crotonin (DCR), a DHC derivative. DHC and DCR (up to 400 μM) inhibited HL60 cell growth, but did not cause serious damage to PBMC. In this study, we found that the ethanol extract from rambutan peel expressed high potential antioxidant activity with no toxicity to normal cells. The peel of this fruit will be a promising source of good antioxidative agents or cancer-preventive agents. However, investigation of the activity associated with further purification, the cultivated conditions and the active constituents of this plant may provide useful comparative information in the future.

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