

ANTIOXIDANT AND CYTOTOXIC ACTIVITIES OF MANGOSTEEN (*GARCINIA MANGOSTANA*) PERICARP EXTRACTS

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Abstract

This study was designed to evaluate the total phenolic compounds (TPC), antioxidation, antiproliferation, and apoptotic properties of mangosteen (*Garcinia mangostana* L.) pericarp ethanolic and water extracts (MPE/e and MPE/w) using the human breast adenocarcinoma cell line, MCF-7, as a model system. The Folin-Ciocalteu method was used for the TPC investigation and DPPH radical scavenging activity and the ferric reducing antioxidant power (FRAP) was used for the antioxidant properties. Cell viability was evaluated by esazurin and 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) assays for 12 and 24 h. The study found that the TPC of the MPE/e were more than those of the MPE/w ($119.95 \pm 1.03 > 84.40 \pm 0.29 \mu\text{g GAE/mg}$, respectively). Consistently, the antioxidant capacity of the MPE/e was significantly higher than that of the MPE/w by about twofold ($P < 0.01$), which was expressed via free radical scavenging with the IC₅₀ of 1199.85 ± 47.16 and $2435.71 \pm 273.74 \mu\text{g/ml}$, and the FRAP with the IC₅₀ of 546.98 ± 26.12 and $1,088.70 \pm 81.54 \mu\text{g/ml}$, respectively. Moreover, only the MPE/e was expressed as a partial inhibitor on the proliferation of MCF-7 cells in a dose- and time-dependent manner. The MPE/e was a strongly positive influence on the antiproliferation and apoptotic induction of the MCF-7 cells, as seen via the morphological changes and oligonucleosomal DNA fragments. In conclusion, the MPE/e may be proposed to develop into cytotoxic or antitumor agents, if it has been investigated that it is effective and non-toxic to the normal cells of animals and humans.

Keywords: Mangosteen, antioxidant activity, antiproliferation, apoptosis

Introduction

Breast cancer is the most commonly diagnosed cancer in women and is the second highest cause of cancer deaths (Merril and Weed, 2001). The development of a new therapeutic approach against breast cancer still remains as one of the

most challenging cancer researches, especially one from natural products.

There are many tropical plants that play a role in biological activities with potential therapeutic applications. Mangosteen (*Garcinia*

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mangostana L.), in the family Guttiferae, is widely cultivated in subtropical regions such as the Philippines, Malaysia, Indonesia, Burma, and Thailand. Mangosteen has long been used as a traditional medicine to treat many diseases such as diarrhea, abdominal pain, dysentery, infected wounds, chronic ulcers, haemorrhoids, food allergies, arthritis, suppuration, leucorrhoea, and gonorrhea (Pierce, 2003; Suksamran *et al.*, 2003; Leiseisen *et al.*, 2004; Moongkarndi *et al.*, 2004). Moreover, studies have reported that mangosteen has anti-bacteria, anti-inflammation, anti-malaria, anti-tumor, and antioxidation properties (Williams *et al.*, 1995; Sakagami *et al.*, 2005; Mahabusarakam *et al.*, 2006). Especially, the pericarp is enriched with phytochemicals and it contains various xanthone derivatives such as α -mangostin, β -mangostin, and γ -mangostin (Farnsworth and Bunyaphatsara, 1992; Pedraza-Chaverri *et al.*, 2008). Recently, α -mangostin and γ -mangostin have been proved to have the potential for cancer cell treatment such as the anti-proliferation of breast cancer cells SKBR3 (Moongkarndi *et al.*, 2004), and also to be able to induce apoptosis of human leukemia cell lines (Matsumoto *et al.*, 2003). However, the MCF-7 human breast cancer cell line and the mechanism of the anticancer activity of α -mangostin are unclear.

The objectives of this work were to investigate the antioxidation, antiproliferation, and the primary mechanism of the cytotoxic properties of mangosteen pericarp extracts (MPEs) using the MCF-7 human breast cancer cell line as a model. Also, the study compared the ethanolic and water solvents to find the highest potential of the extracts. Hopefully, the successful results will be useful for preliminary study on the inhibition of the human breast cancer cell line, MCF7, in the future.

Materials and Methods

Chemicals

The Folin-Ciocalteu reagent and gallic acid were obtained from Fluka Chemie AG, Buchs, Switzerland. Catechin (CA), epigallocatechin-3-gallate (EGCG), ascorbic acid (AA),

2,2-diphenyl-1-picrylhydrazyl (DPPH), and iron (III) chloride hexahydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$) were purchased from Sigma-Aldrich Corporation, St. Louis, MO, USA. Dulbecco's Modified Eagle's Medium: Nutrient Mixture F-12 (DMEM/F-12), fetal bovine serum (FBS), penicillin/streptomycin, and resazurin were from GIBCO, Invitrogen Corporation, Waltham, MA, USA. The 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) and Hoechst 33342 were purchased from Molecular Probes, Invitrogen Corporation, Waltham, MA, USA. Agarose was from Promega Biotech Ibérica, SL, Madrid, Spain. Ethidium bromide was from Bio-Rad Laboratories, Inc., Hercules, CA, USA. RNase A was purchased from Amresco LLC, Solon, OH, USA. The DNA ladder and genomic DNA extraction kit were obtained from RBC Bioscience Corp., Taipei, Taiwan. The Ethidium bromide was from Bio-Rad Laboratories, Inc., Hercules, OH, USA. All other chemicals and reagents were of analytical grade.

Plant Material and Extract Preparation

Mangosteen fruits were collected from Klung sub-district, Klung district, Chanthaburi Province, Thailand. The pericarp was separated from the fruits and then was cleaned, dried, and ground to a powder. The pericarp powder was extracted in 70% ethanol or water for 24 h in a Soxhlet extraction apparatus, and then was evaporated, lyophilized, and kept at -20°C for further study.

Determination of Total Phenolic Compounds

The total phenolic compounds (TPC) were measured by the Folin-Ciocalteu method (Singleton *et al.*, 1999). To obtain a determining concentration, the extract was dissolved with the same solvent as the extraction. One hundred microliters of the sample was mixed with 2 ml of 2% sodium carbonate solution containing 100 μl Folin-Ciocalteu reagent (Folin-Ciocalteu: methanol, 1 : 1, v/v), allowed to stand for 30 min, and then the absorbance was measured at 760 nm. The TCP content was expressed as a microgram of gallic acid equivalents (GAE) per equivalent milligram of sample, using a standard curve generated with gallic acid.

Free Radical Scavenging Activity

The antioxidant activity of the extract was determined by a DPPH (2,2-diphenyl-1-picrylhydrazyl) assay (Sanchez-Moreno *et al.*, 2003). Various concentrations of a 50 μ l sample were mixed with 1.95 ml of fresh DPPH reagent. The mixture was kept in the dark for 45 min and then the absorbance was measured at 515 nm. The AA, CA, and EGCG served as positive controls. The antioxidant activity of the sample was calculated using the following formula and expressed as the median inhibition concentration, IC_{50} .

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

where A_0 was the absorbance of a control, A_1 was the absorbance of the DPPH solution in the presence of a sample, and A_2 was the absorbance without the DPPH solution.

Ferric Reducing Antioxidant Power (FRAP) Assay

The FRAP was determined according to the method of Benzie and Strain (Benzie and Strain, 1996). Briefly, a sample solution of 100 μ l was mixed with 2.9 ml of fresh FRAP reagent, containing 100 mM acetate buffer with pH 3.6, 10 mM TPTZ (2,4,6-tripyridyl-s-triazine), and 20 mM $FeCl_3 \cdot 6H_2O$ solution. The reaction was incubated at 37°C for 30 min and the absorbance measured at 593 nm. The CA and EGCG were used as a standard control. The FRAP value was calculated and expressed as IC_{50} using AA equivalents per μ g of the sample.

Cell Culture

The human breast adenocarcinoma cell line, MCF-7, was obtained from ATCC (American Type Culture Collection), and grown in DMEM/F-12 supplemented with 10% FBS and 1% penicillin/streptomycin in a 5% CO_2 atmosphere at 37°C.

Cell Proliferation Assay

The MCF-7 cell proliferation was determined by measuring the metabolism of tetrazolium substrate MTT. MCF-7 cells at

10000 cells/well in 100 μ l were plated onto a 96-well plate and incubated for 24 h. The medium was then discarded and replaced with the extracts at various concentrations and the incubation continued for 12 and 24 h. Then, the medium was removed and incubated with 100 μ l MTT solution at 5 μ g/ μ l in phosphate-buffered saline (PBS), pH 7.4, for 4 h. 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 absorbance indicated a reduction in cell viability (Okonogi *et al.*, 2007). 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.

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

where A_0 was the absorbance of the control (cells without the extract), A_1 was the absorbance of the treated sample, and A_2 was the absorbance of the treated sample without cells.

Cell Viability Assay

The resazurin assay is a colorimetric method, based on the conversion of the purple resazurin solution to the red resorufin solution via reduction reactions of metabolically active cells. The MCF-7 cells were plated at 10000 cells/well in a 96-well plate and incubated for 24 h. The medium was then discarded and replaced with the extracts at various concentrations and continued to incubate for 12 and 24 h. After that, the medium was removed. The resazurin solution at 100 μ l/ml in DMEM/F-12 without FBS was added to each well and incubated for 2 h. The optical absorbance of the color change was measured at the wavelength of 570 nm with the reference wavelength at 600 nm. Decreasing absorbance indicated a reduction in cell viability (Page *et al.*, 1993; Goegan *et al.*, 1995). The antiproliferation activity (%) was calculated using the following formula and expressed as a median lethal concentration, LC_{50} :

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

where A_0 was the absorbance of the control (cells without the extract), A_1 was the absorbance of the treated sample, and A_2 was the absorbance of the treated sample without cells.

Morphological Observation

Typical nuclear condensation was used as a morphological marker of apoptosis and investigated using Hoechst 33342 dye. The cultured cells were treated with various concentrations of the extract and incubated for 12 and 24 h. The cells were washed twice with ice-cold PBS, pH 7.4. The cells were fixed with 500 μ l formaldehyde (10%, v/v) for 5 min, washed with PBS, and then stained with 1 μ g/ μ l Hoechst 33342 in PBS for 15 min. Cell morphology was observed and photographed under a fluorescence microscope.

DNA Fragmentation Assay

DNA was isolated from the treated MCF-7 cells by using a genomic DNA extraction kit. RNase A at 10 mg/ml was added to sample lysate and left to stand at room temperature for 30 min. The precipitated DNA was dried under centrifuge at 9447 $\times$ g for 3 min and eluted in elution buffer to eluted purified DNA. The DNA sample was quantitated spectrophotometrically and resuspended in TE buffer (10 mM Tris-HCl, pH 7.6, 1 mM EDTA). The DNA at 2 μ g/well was separated in 1% agarose gel containing ethidium bromide (0.5 μ g/ml) in 45 mM Tris, 45 mM boric acid, and 1 mM EDTA at 100 mV for 1.5 h. The gel was visualized under UV fluorescence, and then photographed.

Statistical Analysis

Statistical analysis was performed by ANOVA, using the least significant test to determine the level of significance at $P < 0.05$. For single comparisons, the significance of difference between means was determined by Student's t-test, using the level of significance at $P < 0.05$ and $P < 0.01$. All data were expressed as mean $\pm$ S.E.

Results and Discussion

Total Phenolic Compounds

It has been well known that plant phenolics possess a high antioxidant activity. In this study, the TPC of the MPE/e and MPE/w were 119.95 ± 1.03 and 84.40 ± 0.29 μ g GAE/mg extracts, respectively (Table 1). The TPC of the MPE/e were shown to be significantly higher than those of the MPE/w ($P < 0.05$) that reflected the higher antioxidant activity in the MPE/e compared with the MPE/w.

Mangosteen pericarp contains various phytochemicals, mainly flavonoids, tannin (Ji *et al.*, 2007; Yu *et al.*, 2007), and xanthenes such as 8-desoxygartanin, and α -, β -, and γ -mangostins (Chairungsrilerd *et al.*, 1996; Gopalakrishnan *et al.*, 1997; Huang *et al.*, 2001); however, the chemical sequester is a necessary requirement of specific solvents. In the current study, the TPC of the MPE/e were more than those of the MPE/w ($P < 0.05$). Consistently, some reports revealed that absolute ethanol could sequester more TPC from mangosteen pericarp than 50% ethanol (Wang *et al.*, 2012). Also, Ngawhirunpat *et al.* (2010) found that more α -mangostin in mangosteen pericarp could be extracted by hexane (28.7% w/w) and methanol extracts (15.5% w/w) than by water. That might be because of the water insolubility of α -mangostin. The α - and γ -mangostins are the main chemical components in the xanthenes extract, approximately 81% and 16%, respectively (Aisha *et al.*, 2012). As for the total phenolic and xanthenes contents, they could be extracted by hexane > methanol > water.

Antioxidant Properties

The mangosteen pericarp extracts exhibited proton-donating ability by the DPPH method. In Table 1, the IC_{50} value of the MPE/e (1199.85 ± 47.16 μ g/ml) was significantly lower than that of the MPE/w (2435.71 ± 273.74 μ g/ml) ($P < 0.01$). As a result, the effectiveness of the MPEs was below all the standard antioxidants such as the AA, CA, and EGCG (the IC_{50} values of 110.28 ± 3.93 , 113.85 ± 2.61 , and $63.77 \pm$

1.30 $\mu\text{g/ml}$, respectively). That means the weak effectiveness of the MPEs' activity may involve the TPC.

The presence of reductants (antioxidants) in the MPEs could result in the reduction of the ferric-2,4,6-tri(2-pyridyl)-s-triazine [Fe(III)-TPTZ] complex to its ferrous-2,4,6-tri(2-pyridyl)-s-triazine [Fe(II)-TPTZ]. The MPE/e exhibited a 50% reducing ability with the IC_{50} value of $546.98 \pm 26.12 \mu\text{g/ml}$ which was lower than the MPE/w with the IC_{50} of $1088.70 \pm 81.54 \mu\text{g/ml}$ ($P < 0.01$, Table 1). The FRAP values of the MPEs were higher than those of the CA, with an IC_{50} of $63.36 \pm 1.74 \mu\text{g/ml}$, and the EGCG, with an IC_{50} of $32.37 \pm 1.42 \mu\text{g/ml}$. The result indicated that the MPEs contain electron donors and may relate to the TCP.

In general, the high TPC content is relative to the high antioxidant level which can be seen in many fruit and vegetative species (Leong and Shui, 2002; Chomnawang *et al.*, 2007), the same as in the MPE/e. In the current study, the antioxidant properties of the MPEs were investigated using 2 different antioxidant assays (DPPH and FRAP) based on different principles. The DPPH assay evaluated the ability to quench the synthetic radical DPPH (Blois, 1958). The FRAP assay investigated the antioxidant activity based on the reducing capacity of electron-donating antioxidants (Benzie and Strain, 1996). The current results demonstrated that the MPE/e possessed a higher free radical

scavenging activity and electron-donating capacity than the MPE/w by about twofold, expressed via the IC_{50} values. Methanolic MPE showed a pivotal role in the antioxidant activities via scavenging DPPH, nitric oxide, and lipid radicals in dose-dependent manners (Yoshikawa *et al.*, 1994; Leong and Shui, 2002; Kosem *et al.*, 2007). The α - and γ -mangostins demonstrated antioxidant activity using the ferric thiocyanate method (Fan and Su, 1997). The α -mangostin prevented the decrease of the α -tocopherol consumption induced by low density lipoproteins (LDL) oxidation (Mahabusakam *et al.*, 2000). Consistently, Williams *et al.* (1995) found that α -mangostin inhibited LDL oxidation induced by a copper or peroxy radical.

Antiproliferation Effect on MCF-7 Cells

The human breast adenocarcinoma cell line, MCF-7, were used to investigate the rates of cell proliferation and cell death via the MTT and resazurin methods. The cytoprotective activity of the MPEs on the MCF-7 cells was evaluated after drug treatment for 12 and 24 h. Interestingly, the MPE/e was partially potent against the proliferation of MCF-7 cells. The proliferation potency, the LC_{50} in the MTT method at 12 and 24 h of the MPE/e, were 590.64 ± 8.01 and $430.91 \pm 2.21 \mu\text{g/ml}$, respectively (Table 2). Consistent with the resazurin method, the LC_{50} values at 12 and 24 h of the MPE/e were 670.13

Table 1. Total phenolic compounds of mangosteen pericarp extracts and their antioxidant activities, assessed by DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging and ferric reducing antioxidant power (FRAP). Data were mean $\pm$ SE, n=4

Sample	Total Phenolics ($\mu\text{g GAE/mg}$)	DPPH IC_{50} ($\mu\text{g/ml}$)	FRAP IC_{50} ($\mu\text{g/ml}$)
MPE/e	$119.95 \pm 1.03^*$	$1199.85 \pm 47.16^{c, **}$	$546.98 \pm 26.12^{c, **}$
MPE/w	84.40 ± 0.29	2435.71 ± 273.74^d	1088.70 ± 81.54^d
AA	-	110.28 ± 3.93^b	-
CA	-	113.85 ± 2.61^b	63.36 ± 1.74^b
EGCG	-	63.77 ± 1.30^a	32.37 ± 1.42^a

MPE/e, mangosteen pericarp ethanolic extract; MPE/w, mangosteen pericarp water extract; AA, ascorbic acid; CA, catechin; EGCG, epigallocatechin-3-gallate; IC_{50} , median inhibitory concentration. Superscript alphabets in the same column are significantly different ($P < 0.05$). * $P < 0.05$ and ** $P < 0.01$

± 9.42 and $501.75 \pm 14.07 \mu\text{g/ml}$, respectively (Table 3). Conversely, the MPE/w showed the least effective LC_{50} value in both methods (data not shown). Based on this study, the MPE/e displayed more antiproliferative effects on the MCF-7 cells in a dose- and time-dependent manner; however, the LC_{50} values of the MPE/e were significantly higher than those of the EGCG by about 2.9–3.4 times (Tables 2 and 3).

Antiproliferation of the MCF-7 cancer cells was significantly increased in a dose- and time-dependent fashion of the MPE/e, as seen in an investigation by the MTT and resazurin assays in this study, although the MPE/e had a more antiproliferative effect on the MCF-7 cell inhibition as seen in the value being lower than the EGCG by approximately threefold. Methanolic MPE could significantly inhibit the proliferation of the human umbilical vein endothelial (ECV304) cell line (Kosem *et al.*, 2007) and the human breast cancer (SKBR3) cell line (Moongkarndi *et al.*, 2004). The γ -mangostin exhibited potentially antiproliferative activity toward human malignant glioblastoma cells, including U87 MG and GBM 8401 (Chang *et al.*, 2010). Six xanthenes from mangosteen pericarp exhibited antiproliferative activity against the

human leukemia (HL60) cell line and it was also found that the mechanism of the HL60 cell death was induced by α - and γ -mangostins (Matsumoto *et al.*, 2003; Matsumoto *et al.*, 2004). Consistently, Matsumoto *et al.* (2005) found that α - and γ -mangostins strongly inhibited the human colon cancer DLD-1 cell growth via the cell-cycle arrest and apoptotic process. In recent studies, MPE and γ -mangostin showed a cytotoxic effect on MCF-7 cancer cells and the antiproliferative effect was associated with apoptosis (Yu *et al.*, 2009; Chitra *et al.*, 2010). These results suggest that the MPE/e has a partial inhibitory effect on MCF-7 cells.

Morphological Changes of MCF-7 Cells

After $400 \mu\text{g/ml}$ MPE/e incubation, morphological alterations of the control (untreated cells) and MCF-7 cells are illustrated in Figure 1A and 1B, respectively. As a result, the untreated cells still had a normal polygonal shape (Figures 1 A(1) and A(2), arrow No. 1). But, the MCF-7 cells exposed to the MPE/e for 24 h were induced to retract to a round shape, and some sensitive cells were detached from the surface. The treated cells demonstrated membrane blebbing (Figures 1 B(1) and B(2),

Table 2. Antiproliferative activity of mangosteen pericarp extracts on MCF-7 cells, assessed by MTT assay. Data represents mean $\pm$ SE, $n=4$

Sample	Concentration ($\mu\text{g/ml}$)	12 h		24 h	
		Antiproliferation (%)	LC_{50} ($\mu\text{g/ml}$)	Antiproliferation (%)	LC_{50} ($\mu\text{g/ml}$)
MPE/e	400	6.30 ± 1.03	$590.64 \pm 8.01^{\text{b}, \text{b}}$	51.28 ± 2.37	$430.91 \pm 2.21^{\text{b}, \text{a}}$
	500	34.61 ± 3.45		76.85 ± 0.82	
	600	51.32 ± 1.24		80.70 ± 1.77	
MPE/w	500	3.22 ± 0.51	n/a	5.05 ± 0.98	n/a
	1000	5.65 ± 1.04		8.20 ± 1.34	
	2000	10.10 ± 0.54		13.20 ± 1.74	
EGCG	100	6.75 ± 1.72	$163.36 \pm 1.32^{\text{a}, \text{b}}$	15.46 ± 1.27	$148.84 \pm 1.93^{\text{a}, \text{a}}$
	150	46.94 ± 3.21		56.96 ± 2.22	
	200	70.82 ± 1.18		79.85 ± 1.06	

MPE/e, mangosteen pericarp ethanolic extract; MPE/w, mangosteen pericarp water extract; EGCG, epigallocatechin-3-gallate; n/a, not available; LC_{50} , median lethal concentration. The symbol "^a...." and "...^a" refer to the comparison between and within groups, respectively.

arrow No. 2) and an apoptotic body (Figure 1 B(2), arrow No. 3). Staining with the DNA probe Hoechst 33342 produced a distinct nuclear morphology of apoptotic cells, which were stained with or without nuclear shrinking

and nuclear fragmentation. However, the non-apoptotic cells exhibited uniformly stained nuclei (Figure 1(a3)). In addition, the MPE/e-treated cells illustrated nuclear shrinking (Figure 1(b3), arrow No. 4) and nuclear

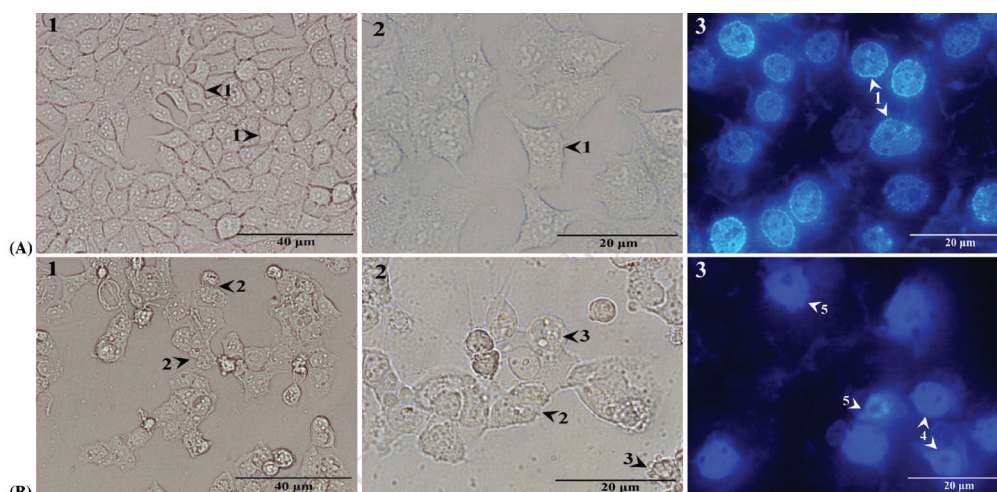


Figure 1. Morphological alterations of MCF-7 cells following exposure to 400 µg/ml of MPE/e for 24 h. Control MCF-7 cells were observed under inverted microscopy at 200x and 400x magnifications (A1-2) and staining with Hoechst 33342 (A3). MPE/e-treated MCF-7 cells were observed under inverted microscopy at 200x and 400x magnifications (B1-2) and staining with Hoechst 33342 (B3). The numbers represent to 1 > : normal cells; 2 > : membrane blebbing; 3 > : apoptotic body; 4 > : nuclear shrinking; and 5 > : nuclear fragmentation

Table 3. Antiproliferative activity of mangosteen pericarp extracts on MCF-7 cells, assessed by resazurin assay. Data represents mean $\pm$ SE, n=4

Sample	Concentration (µg/ml)	12 h		24 h	
		Antiproliferation (%)	LC ₅₀ (µg/ml)	Antiproliferation (%)	LC ₅₀ (µg/ml)
MPE/e	400	11.96 $\pm$ 1.63	670.13 $\pm$ 9.42 ^{b, b}	32.02 $\pm$ 0.54	501.75 $\pm$ 14.07 ^{b, a}
	500	25.84 $\pm$ 1.03		48.19 $\pm$ 2.41	
	600	41.69 $\pm$ 2.26		85.36 $\pm$ 2.50	
MPE/w	500	1.42 $\pm$ 0.28	n/a	2.90 $\pm$ 0.63	n/a
	1000	1.95 $\pm$ 0.48		5.19 $\pm$ 0.27	
	2000	2.92 $\pm$ 0.66		9.92 $\pm$ 0.59	
EGCG	100	2.16 $\pm$ 0.39	194.60 $\pm$ 7.58 ^{a, b}	13.45 $\pm$ 1.20	152.78 $\pm$ 1.16 ^{a, a}
	150	27.34 $\pm$ 3.93		56.40 $\pm$ 2.13	
	200	53.75 $\pm$ 3.37		75.10 $\pm$ 1.88	

MPE/e, mangosteen pericarp ethanolic extract; MPE/w, mangosteen pericarp water extract; EGCG, epigallocatechin-3-gallate; n/a, not available; LC₅₀, median lethal concentration. The symbol ^a and ^b refer to the comparison between and within groups, respectively.

fragmentation (Figure 1(b3), arrow No. 5). This study indicated that the MPE/e could induce apoptosis, as seen from the morphological feature.

Morphological changes and DNA fragmentation patterns on the agarose gel electrophoresis are used to investigate apoptosis of the MCF-7 breast cancer cells caused by the MPE/e manner. In this study, the MPE/e was able to induce MCF-7 cell morphological changes which were a cause of DNA fragmentation in a dose- and time-dependent manner. The changes were cytoplasmic membrane shrinkage, loss of contact with neighboring cells, membrane blebbing, and an apoptotic body, as seen via an inverted microscopic investment (Figure 1(b)). However, after 400 $\mu\text{g/ml}$ MPE/e treatment for 24 h and staining with Hoechst 33342, the morphological cells had been changed, as seen via nuclear shrinking, DNA condensation, and fragmentation (Figure 1(b)). In addition, DNA fragmentation was demonstrated by 1% agarose gel electrophoresis (Figure 2) after 300-500 $\mu\text{g/ml}$

of MPE/e treatment for 12 and 24 h. These recognized features of morphological changes and DNA fragmentation suggest that the MPE/e induces apoptosis of the MCF-7 cells.

DNA Fragmentation of MCF-7 Cells

The DNA fragmentation of MCF-7 cells was detected by agarose gel electrophoresis after 0, 300, 400, and 500 $\mu\text{g/ml}$ of MPE/e treatment for 12 and 24 h. The results showed that all those MPE/e concentrations could induce dose- and time- dependent DNA fragmentation in MCF-7 cells (Figure 2), while the control did not. Therefore, it is possible that the effector caspases executed the apoptotic signal stimulated by the treatment compound.

It is well known that apoptosis can occur by 2 major pathways; the extrinsic pathway involving the death receptor signaling and the intrinsic pathway involving the mitochondrial cascades (Henry-Mowatt *et al.*, 2004). The extrinsic pathway is involved with caspase-8, and the intrinsic pathway is associated with

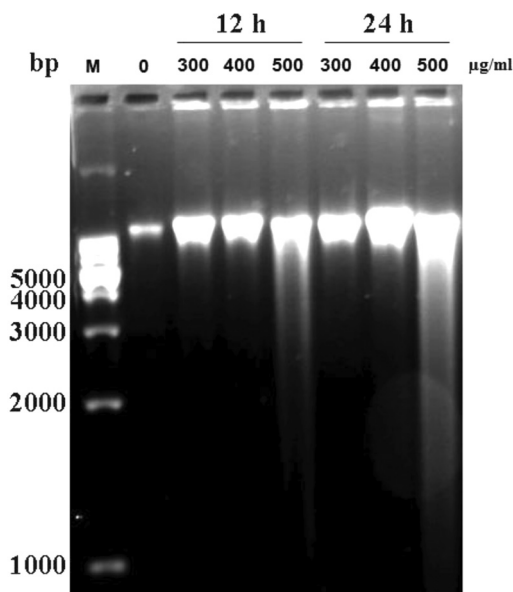


Figure 2. DNA fragmentation in MPE/e-treated MCF-7 cells at designated concentrations and times. The lanes represent different meanings: DNA marker (lane M); control MCF-7 cells (Lane 1); MPE/e-treated MCF-7 cells at 300-500 $\mu\text{g/ml}$, 12 h (Lane 2-4); MPE/e-treated MCF-7 cells at 300-500 $\mu\text{g/ml}$, 24 h. (Lane 5-7)

caspase-9 (Ashkenazi *et al.*, 2000). Activation of caspase-3/7 (effector caspases) is involved in both pathways. Matsumoto *et al.* (2004) revealed that α - and γ -mangostins induced apoptosis in human colon cancer DLD-1 cells. Aisha *et al.* (2012) found that xanthones extract of mangosteen pericarp and α -mangostin showed potent cytotoxicity due to the induction of the mitochondria pathway of apoptosis in HCT116 colorectal carcinoma. In addition, MPE induced apoptosis in SKBR3 cells by investigation of morphological changes and oligonucleosomal DNA fragments (Moongkarndi *et al.*, 2004). Consistently, α -mangostin induced apoptosis in HL60 cells, which was mediated by mitochondrial dysfunctions in the early phase. They found that this xanthone induced caspase -9 and -3 activation, loss of mitochondrial membrane potential, and release of reactive oxygen species and cytochrome C (Matsumoto *et al.*, 2005). Moreover, the MPE/e induced apoptosis via caspase activation (caspase-3/7, 8, and 9 activities), increased the Bax/Bcl-2 ratio, cytochrome c release, and decreased Akt1 on human squamous cell carcinoma A-431 and melanoma SK-MEL-28 lines (Wang *et al.*, 2012). The recent study showed that mangostin and xanthone increased the protein kinase A activity level but decreased the expression of the estrogen receptor. Mangostin and xanthone could induce cell death of MCF-7 cells through the inhibition of the mitogen-activated protein kinases pathway (Tan and Sophianie, 2011). These findings indicate that MPEs which induce apoptosis may work by several mechanisms.

Conclusions

This study found that the MPE/e and MPE/w had low antioxidant activities when compared with the standard antioxidants such as ascorbic acid, catechin, and epigallocatechin-3-gallate. However, only the MPE/e had a partial antiproliferation against MCF-7 cells by inducing apoptotic cell death in a dose- and time-dependent manner. The results from this study could propose that the MPE/e may be worth further study on the cytotoxicity of normal human cells. Some constituents of the MPE/e

may serve as novel powerful cytotoxic agents and may have an effect on other cell lines as well. It is suggested that the MPE/e should be further investigated for toxicity to normal cell lines in both animals and humans.

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