



Antioxidant and Cytotoxic Activities of Mangosteen *Garcinia mangostana* Pericarp Extracts

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Abstract

This study was designed to evaluate the total phenolic content (TPC), antioxidation, antiproliferation and apoptotic properties of mangosteen (*Garcinia mangostana* L.) pericarp ethanolic and water extracts (MPE/e and MPE/w) using human breast adenocarcinoma cell line, MCF-7, as a model system. Folin-Ciocalteu method was used for TPC analysis and DPPH as well FRAP were used for antioxidant capacity. Cell viability was evaluated by Resazurin and 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) assays for 12 and 24 h. The study found that TPC of MPE/e was over than MPE/w ($119.95 \pm 1.03 > 84.40 \pm 0.29$ $\mu\text{g GAE/mg}$, respectively). Consistently, the antioxidant capacity of MPE/e was higher than MPE/w about 2 folds, which expressed via free radical scavenging, IC_{50} of $1,199.85 \pm 47.16$ and $2,435.71 \pm 273.74$ $\mu\text{g/ml}$ and FRAP, IC_{50} of 546.98 ± 26.12 and $1,088.70 \pm 81.54$ $\mu\text{g/ml}$. Moreover, only MPE/e expressed as a potential inhibitor on the MCF-7 cells proliferation at dose- and time- dependent manner. MPE/e was strongly positive influence on antiproliferation and apoptotic induction of MCF-7 cells as seen via the morphological changes and oligonucleosomal DNA fragments. In conclusion, MPE/e may be proposed to develop to cytotoxic agents or antitumor if it has been investigated that effective and non-toxic to normal cells of animals and humans.

Keywords : *Garcinia mangostana*, antioxidant activity, antiproliferation, apoptosis

1. Introduction

Breast cancer is the most commonly diagnosed cancer in women and is the second highest cause of cancer death (1). The development of new therapeutic approach against breast cancer still remains as one of the most challenging cancer researches, especially from natural products.

There are many tropical plants play a role on biological activities with potential therapeutic applications. Mangosteen (*Garcinia mangostana* L.), family Guttiferae, is widely cultivated in subtropical regions such as Philippines, Malaysia, Indonesia, Burma and Thailand. Mangosteen has long been used as a traditional medicine to treat many diseases such as for diarrhea, abdominal

pain, dysentery, infected wounds, chronic ulcer, haemorrhoids, food allergies, arthritis, suppuration, leucorrhoea and gonorrhoea (2-4). Moreover, the studies reported that mangosteen has anti-bacteria, anti-inflammation, anti-malaria, anti-tumor and antioxidation (5-7). Especially, the pericarp enriches of phytochemicals and it contains various xanthone derivatives such as α -mangostin, β -mangostin, and γ -mangostin (8). Recently, α -mangostin and γ -mangostin have been proved that they have potentially on cancer cell treatment such as anti-proliferation on breast cancer cells SKBR3 (4), and also could induce apoptosis of human leukemia cell lines (9). However, MCF-7 human breast cancer cell line and the mechanism of anticancer activity of α -mangostin is unclear.

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

2. Materials and Methods

2.1 Chemicals

Folin-Ciocalteu reagent and gallic acid obtained from Fluka Chemie AG, 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, 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, USA. 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) and Hoechst 33342 were purchased from Molecular Probes, Invitrogen Corporation, USA. Agarose was from Promega, Spain. Ethidium bromide was from Bio-Rad, USA. RNase A was purchased from Amresco[®], USA. DNA ladder and Genomic DNA Extraction Kit were obtained from RBC Bioscience, USA. All other chemicals and reagents were of analytical grade. Ethidium bromide was purchased from Bio-Rad, USA.

2.2 Plant material and extract preparation

Mangosteen fruits were collected from local farms in Chanthaburi Province, Thailand. Pericarp was separated from fruits and then was cleaned, dried and ground to 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.

2.3 Determination of total phenolic content

Total phenolic content (TPC) were measured by Folin-Ciocalteu method. To obtain a determining concentration, the extract was dissolved with the same solvent of the extraction. One hundred microliters of 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 measured the absorbance at 760 nm. TPC was expressed as microgram of gallic acid equivalents (GAE) per milligram of sample, using a standard curve generated with gallic acid.

2.4 Free radical scavenging activity

The antioxidant activity of the extract was determined by DPPH[•] (2,2-diphenyl-1-picrylhydrazyl) assay. Various concentrations of 50 µl 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. Ascorbic acid (AA), catechin (CA) and epigallocatechin-3-gallate (EGCG) were served as positive controls. The antioxidant activity of sample was calculated using the following formula and expressed as median inhibition concentration, IC₅₀.

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

Where A₀ was the absorbance of the control (DPPH[•] solution without sample), A₁ was the absorbance of DPPH[•] solution in the presence of a sample and A₂ was the absorbance without DPPH[•] solution.

2.5 Ferric reducing antioxidant power (FRAP) assay

The ferric reducing antioxidant power (FRAP) was determined according to the method of Benzie and Strain (10). Briefly, sample solution of 100 µl was mixed with 2.9 ml of fresh FRAP reagent, containing 100 mM acetate buffer, pH 3.6, 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) and 20 mM FeCl₃·6H₂O solution. The reaction was incubated at 37 °C for 30 min and measured the absorbance at 593 nm. CA and EGCG were used as a standard control. The FRAP value was calculated and expressed as IC₅₀ using AA equivalents per µg of sample.

2.6 Cell culture

Human breast adenocarcinoma cell line, MCF-7 cells, obtained from American Type Culture Collection; ATCC, was

grown in DMEM/F-12 supplemented with 10% FBS and 1% penicillin/streptomycin in a 5% CO₂ atmosphere at 37 °C.

2.7 Cell proliferation assay

MCF-7 cell proliferation was determined by measuring the metabolism of tetrazolium substrate MTT. MCF-7 cells at 10,000 cells/well in 100 µl 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 continued to incubate for 12 and 24 h. Then, medium was removed and incubated with 100 µl MTT solution at 5 µg/µl 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 in absorbance indicated a reduction in cell viability. The antiproliferation activity (%) was plotted against the sample concentrations and the median lethal concentration of 50% (LC₅₀) was derived from the best fit line obtained by linear regression analysis.

$$\text{Antiproliferation activity (\%)} = \left[1 - \frac{(A_1 - A_2)}{(A_0 - A_2)} \right] \times 100 \dots (\text{eq. 2})$$

Where A₀ was the absorbance of the control (cells without the extract), A₁ was the absorbance of the treated sample and A₂ was the absorbance of treated sample without cells.

2.8 Cell viability assay

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 10,000 cells/well in 96-well plate and incubated for 24 h. Then, the medium was discarded and replaced by the determining extract concentrations. After continually incubated for 12 and 24 h, the medium was removed. 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 in absorbance indicated a reduction in cell viability. The percentage of antiproliferative effect was calculated as following to the equation no.2 and expressed as median lethal concentration, LC₅₀.

2.9 Morphological observation

Typical nuclear condensation was used as a morphological marker of apoptosis and observed using Hoechst 33342 dye. The cultured cells were treated with various concentrations of 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 fluorescent microscope.

2.10 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 13,000 rpm for 3 min and eluted in elution buffer. The DNA sample was quantitated spectrophotometrically and resuspended in TE buffer (10 mM Tris-HCl, pH 7.6, 1 mM EDTA). 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, 1 mM EDTA at 100 mV for 1.5 h. The gel was visualized under UV fluorescence, and then photographed.

2.11 Statistical analysis

Statistical analysis was performed by T-test, using the least significant test to determine the level of significant at $P < 0.05$ and $P < 0.01$. All data were expressed as mean \pm SE.

3. Results and Discussion

3.1 Total phenolic content

It has been well known that plant phenolic possess high antioxidant activity. In this study, the TPC of mangosteen pericarp ethanolic and water extracts (MPE/e and MPE/w) were 119.95 ± 1.03 and 84.40 ± 0.29 μ g GAE/mg extracts, respectively (Table 1). TPC of MPE/e substantially exhibited significantly higher than that of MPE/w ($P < 0.05$) that referred to high antioxidant activity in MPE/e over than MPE/w.

Mangosteen pericarp contained various phytochemicals, mainly flavonoids, tannin (11-12) and xanthones such as 8-desoxygartanin, and α -, β -, and γ -mangostins (13-14); however, the chemical sequester is necessary requirement of specific solvents. In the current study, TPC of MPE/e was over than that of MPE/w ($P < 0.05$). Consistently, some reports revealed that absolute ethanol could sequester more TPC from mangosteen pericarp than 50% ethanol (15). Also, Ngawhirunpat *et al.* (16) found that α -mangostin in mangosteen pericarp could be extracted in higher amounts by hexane (28.7% w/w) and methanol extracts (15.5% w/w) than by water. That might be because of water insolubility of α -mangostin. The α - and

γ -mangostins are mainly chemical components in xanthenes extract, approximately 81% and 16%, respectively (17). As for

total phenolic and xanthenes contents, they could be extracted by hexane > methanol > water.

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).

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*	1,199.85 \pm 47.16**	546.98 \pm 26.12**
MPE/w	84.40 \pm 0.29	2,435.71 \pm 273.74	1,088.70 \pm 81.54
AA	-	110.28 \pm 3.93	-
CA	-	113.85 \pm 2.61	63.36 \pm 1.74
EGCG	-	63.77 \pm 1.30	32.37 \pm 1.42

MPE/e, mangosteen pericarp ethanolic extract; MPE/w, mangosteen pericarp water extract; AA, ascorbic acid; CA, catechin; EGCG, epigallocatechin-3-gallate; IC₅₀, median inhibitory concentration. * P < 0.05 and ** P < 0.01. Data were mean \pm SE, n=4.

3.2 Antioxidant properties

The mangosteen pericarp extracts exhibited proton-donating ability by DPPH method. In Table 1, IC₅₀ value of MPE/e (1,199.85 \pm 47.16 $\mu\text{g/ml}$) was significantly lower than that of MPE/w (2,435.71 \pm 273.74 $\mu\text{g/ml}$) (P < 0.01). However, the effectiveness of MPEs was below than all standard antioxidants tested i.e. AA, CA and EGCG showing the IC₅₀ values of 110.28 \pm 3.93, 113.85 \pm 2.61, and 63.77 \pm 1.30 $\mu\text{g/ml}$, respectively. That means the weak effectiveness of MPEs activity may involve to TPC.

The presence of reductants (antioxidants) in MPEs could result in the reduction of 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]. MPE/e exhibited 50% reducing ability with IC₅₀ value of 546.98 \pm 26.12 $\mu\text{g/ml}$ lower than MPE/w with IC₅₀ of 1,088.70 \pm 81.54

$\mu\text{g/ml}$ (P < 0.01, Table 1). The FRAP values of MPEs were higher than that of CA (IC₅₀ of 63.36 \pm 1.74 $\mu\text{g/ml}$) and EGCG (IC₅₀ of 32.37 \pm 1.42 $\mu\text{g/ml}$). The result indicated that MPEs contain electron donors and may relate to TPC.

In general, high TPC is relative to high antioxidant level which could be seen in many fruit and vegetative species (18) as same as in MPE/e. In the current study, the antioxidant properties of MPEs were investigated using two different antioxidant assays (DPPH and FRAP) based on different principles. DPPH assay evaluated the ability to quench the synthetic radical DPPH. FRAP assay investigated the antioxidant activity based on the reducing capacity of electron-donating antioxidants. The current results demonstrated that MPE/e possessed higher free radical scavenging activity and electron-donating capacity than MPE/w for about 2 folds,

expressing via IC_{50} values. Methanolic MPE showed a pivotal role on the antioxidant activities via scavenging DPPH, nitric oxide and lipid radicals in dose-dependent manners (18-20). The α - and γ -mangostins demonstrated antioxidant activity using the ferric thiocyanate method (21). The α -mangostin prevented the decrease of the α -tocopherol consumption induced by LDL oxidation (22). Consistently, William et al. (5) found that α -mangostin inhibited low density lipoproteins (LDL) oxidation inducing by copper or peroxy radical.

3.3 Antiproliferation effect on MCF-7 cells

Human breast adenocarcinoma cell line, MCF-7 cells, were used to investigate the rates of cell proliferation and cell viability via MTT and Resazurin methods. The cytoprotective activity of MPEs on MCF-7 cells was evaluated after sample treatment for 12 and 24 h. Interestingly, MPE/e was partially potent against the proliferation of MCF-7 cells. The proliferation potency, LC_{50} in MTT method at 12 and 24 h of MPE/e were 590.64 ± 8.01 and 430.91 ± 2.21 $\mu\text{g/ml}$, respectively (Table 2). Consistent with the Resazurin method, the LC_{50} values at 12 and 24 h of MPE/e were 670.13 ± 9.42 and 501.75 ± 14.07 $\mu\text{g/ml}$, respectively (Table 3). In the opposite, MPE/w showed the least effective of LC_{50} value in both methods (data not shown). Based on this study, MPE/e played more antiproliferative effects on MCF-7 cells as dose- and time-dependent manner. However, LC_{50} values of MPE/e was significantly higher than that of EGCG about 2.9-3.4 folds (Tables 2 and 3).

Antiproliferation of MCF-7 cancer cells was significantly increased as dose- and time-dependent fashion of MPE/e as seen from MTT and Resazurin assays in

this study. Although, MPE/e was more antiproliferative effect on MCF-7 cells inhibition as seen the value lowering than EGCG approximately 3 folds. Methanolic MPE could significantly inhibit the proliferation of human umbilical vein endothelial (ECV304) cell line (20) and human breast cancer (SKBR3) cell line (4). The γ -mangostin exhibited potentially antiproliferative activity toward human malignant glioblastoma cells, including U87 MG and GBM 8401 (23). Six xanthenes from mangosteen pericarp exhibited antiproliferative activity against human leukemia (HL60) cell line and the mechanism of HL60 cell death induced by α - and γ -mangostins (9, 24). Consistently, Matsumoto *et al.* (25) found that α - and γ -mangostins strongly inhibited human colon cancer DLD-1 cell growth via cell-cycle arrest and apoptotic process. In the recent study, MPE and γ -mangostin showed cytotoxic effect on MCF-7 cancer cells and the antiproliferative effect was associated with apoptosis (26-27). These results suggest that MPE/e has a partial inhibitory effect on MCF-7 cells.

3.4 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 were illustrated in Figure 1A and 1B, respectively. As a result, the untreated cells still had normal shape with polygonal (Figures 1 A(1) and A(2), arrow No. 1). But, the MCF-7 cells exposing with MPE/e for 24 h were induced to retraction and rounding shape, and some sensitive cells were detached from the surface. The treated cells demonstrated membrane blebbing (Figures 1 B(1) and B(2), arrow No. 2) and 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 A(3)). In addition, the MPE/e-treated cells were illustrated nuclear shrinking (Figure 1 B(3), arrow No. 4) and nuclear fragmentation (Figure 1 B(3), arrow No. 5). This study indicated that MPE/e could induce apoptosis as seen from the morphological feature.

Morphological changes and DNA fragmentation patterns on agarose gel electrophoresis are used to investigate apoptosis of MCF-7 breast cancer cells causing by MPE/e manner. In this study, MPE/e was able to induce MCF-7 cell morphological changes which were a cause

of DNA fragmentation as dose- and time-dependent manner. The changings were cytoplasmic membrane shrinkage, loss of contact with neighboring cells, membrane blebbing and 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 suggested that MPE/e induced apoptosis of MCF-7 cells.

Table 2. Antiproliferative activity of mangosteen pericarp extracts on MCF-7 cells, assessed by MTT assay.

Sample	Conc. ($\mu\text{g/ml}$)	12 h		24 h	
		Antiproliferation (%)	LC ₅₀ ($\mu\text{g/ml}$)	Antiproliferation (%)	LC ₅₀ ($\mu\text{g/ml}$)
MPE/e	400	6.30 \pm 1.03	590.64 \pm 8.01	51.28 \pm 2.37	430.91 \pm 2.21
	500	34.61 \pm 3.45		76.85 \pm 0.82	
	600	51.32 \pm 1.24		80.70 \pm 1.77	
MPE/w	500	3.22 \pm 0.51	n/a	5.05 \pm 0.98	n/a
	1,000	5.65 \pm 1.04		8.20 \pm 1.34	
	2,000	10.10 \pm 0.54		13.20 \pm 1.74	
EGCG	100	6.75 \pm 1.72	163.36 \pm 1.32	15.46 \pm 1.27	148.84 \pm 1.93
	150	46.94 \pm 3.21		56.96 \pm 2.22	
	200	70.82 \pm 1.18		79.85 \pm 1.06	

MPE/e, mangosteen pericarp ethanolic extract; MPE/w, mangosteen pericarp water extract; EGCG, epigallocatechin-3-gallate; LC₅₀, median lethal concentration. Data represents mean \pm SE, n=4.

Table 3. Antiproliferative activity of mangosteen pericarp extracts on MCF-7 cells, assessed by Resazurin assay.

Sample	Conc. ($\mu\text{g/ml}$)	12 h		24 h	
		Antiproliferation (%)	LC ₅₀ ($\mu\text{g/ml}$)	Antiproliferation (%)	LC ₅₀ ($\mu\text{g/ml}$)
MPE/e	400	11.96 \pm 1.63	670.13 \pm 9.42	32.02 \pm 0.54	501.75 \pm 14.07
	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
	1,000	1.95 \pm 0.48		5.19 \pm 0.27	
	2,000	2.92 \pm 0.66		9.92 \pm 0.59	
EGCG	100	2.16 \pm 0.39	194.60 \pm 7.58	13.45 \pm 1.20	152.78 \pm 1.16
	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; LC₅₀, median lethal concentration. Data represents mean \pm SE, n=4.

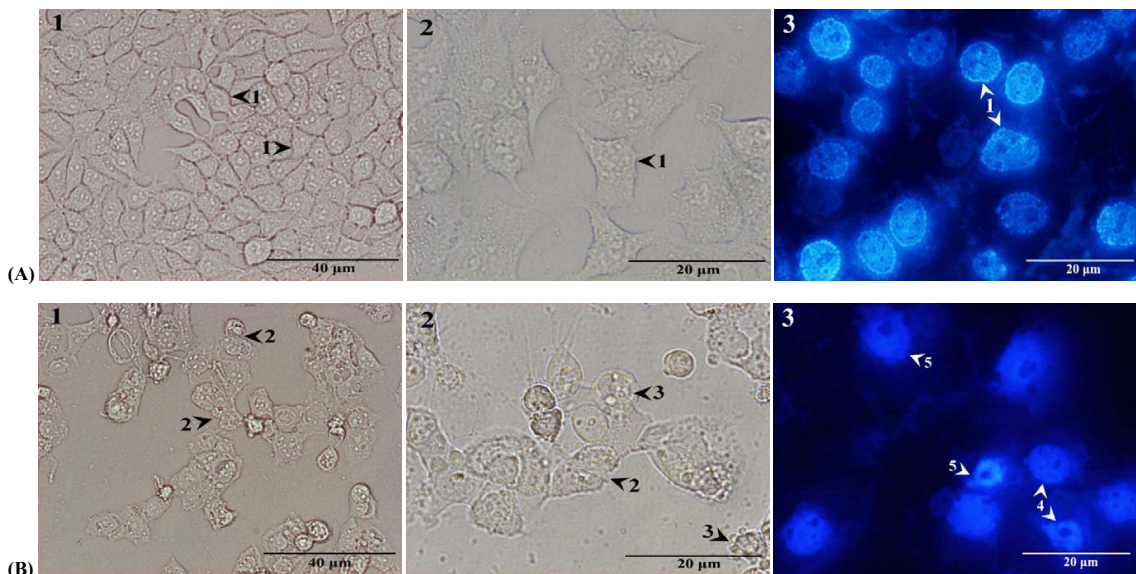


Figure 1. Morphological alterations of MCF-7 cells following exposure to 400 $\mu\text{g/ml}$ of MPE/e for 24 h. Control MCF-7 cells were observed under inverted microscopy at 200x and 400x magnifications (A1-2) and staining with Hoechst 33342 (A3). MPE/e-treated MCF-7 cells were observed under inverted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

3.5 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 to dose- and time- dependent DNA fragmentation in MCF-7 cells (Figure 2), while the control did not have. Therefore, it is possible that the effector caspases executed the apoptotic signal stimulated by the treatment compound.

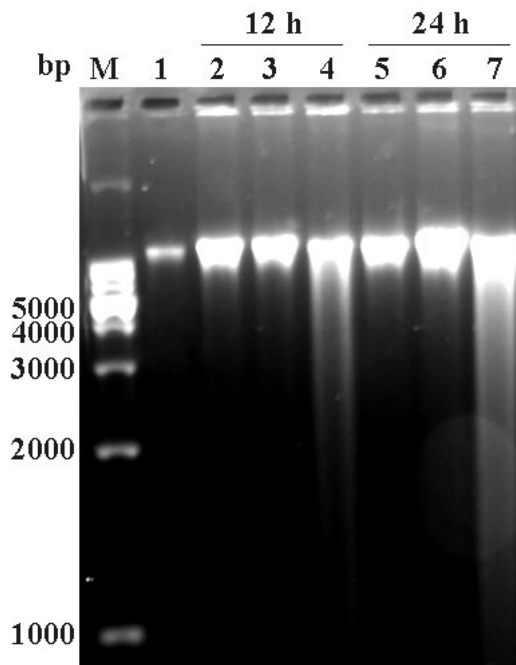


Figure 2. DNA fragmentation in MPE/e-treated MCF-7 cells at designated concentrations and times. The lanes represent different meanings: DNA marker (lane M); control MCF-7 cells (Lane 1); MPE/e-treated MCF-7 cells at 300-500 $\mu\text{g/ml}$, 12 h (Lane 2-4); MPE/e-treated MCF-7.

It is well known that apoptosis can occur by two major pathways; the extrinsic pathway involving the death receptor signaling and the intrinsic pathway involving the mitochondrial cascades (28). The extrinsic pathway is involved in caspase-8, and the intrinsic pathway is associated with caspase-9 (29). Activation of caspase-3/7 (effector caspases) is involved in both pathways. Matsumoto et al. (24) revealed

that α - and γ -mangostins induced apoptosis in human colon cancer DLD-1 cells. Aisha et al. (17) found that xanthenes extract of mangosteen pericarp and α -mangostin showed potent cytotoxicity and due to induction of the mitochondria pathway of apoptosis on HCT116 colorectal carcinoma. In addition, MPE induced apoptosis on SKBR3 cells by showing morphological changes and oligonucleosomal DNA

fragments (4). Consistently, α -mangostin induced apoptosis in HL60 cells, which was mediated by mitochondrial dysfunctions in the early phase. Matsumoto et al. (25) found that this xanthone induced caspase-9 and -3 activation, loss of mitochondrial membrane potential, and release of ROS and cytochrome C. Moreover, MPE/e induced apoptosis via caspase activation (caspase-3/7, 8 and 9 activities), increased Bax/Bcl-2 ratio, cytochrome c release, and decreased Akt1 on human squamous cell carcinoma A-431 and melanoma SK-MEL-28 lines (15). The recent study showed that mangostin and xanthone increased protein kinase A (PKA) activity level but decreased the expression of estrogen receptor (ER). Mangostin and xanthone could induce cell death of MCF-7 cells through the inhibition of MAPK pathway (30). These findings indicate that MPE which induce apoptosis may work by several mechanisms.

4. Conclusion

In this study, we found that MPE/e and MPE/w have low antioxidant activities when comparing with the standard antioxidants such as ascorbic acid, catechin and epigallocatechin-3-gallate. However, only MPE/e had a powerful antiproliferative effect against MCF-7 cells by inducing apoptotic cell death at dose- and time-dependent manner. The results from this study could be proposed that MPE/e may be the worth for further study on cytotoxic of human normal cells. Some constituents of MPE/e may serve as a novel powerful cytotoxic agents and may effect on other cell lines as well. In suggestion, MPE/e should be further investigated toxicity in normal cell lines, animals and humans.

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