EFFECTS OF MANGOSTEEN HULL EXTRACTS ON BIOEFFICACY AND ANTIPROLIFERATION OF HUMAN BREAST AND PROSTATE CARCINOMA CELL LINES

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Abstract

This study aimed to investigate the benefits of methanolic and ethanolic mangosteen (*Garcinia mangostana* L.) hull extracts (MHE/m and MHE/e) for medicinal properties. The results found that MHE/m contained total phenolic compounds and flavonoids contents greater than MHE/e. Also, the free radical scavenging activity and lipid peroxidative inhibition of MHE/m were higher than those of MHE/e, but the values were significantly lower than the antioxidant standards such catechin (CA) and epigallocatechin-3-gallate (EGCG). The study of antiproliferation on human MCF-7 breast and PC-3 prostate cancer cell lines via MTT and the resazurin method found that the MHEs could inhibit both cancer cell lines better than CA, but worse than EGCG. As for the cytotoxicity test via brine shrimp and mosquito larvae lethality assays, the MHEs were more toxic on the brine shrimp and mosquito larvae. In conclusion, it indicates that MHEs can exhibit different activities and have the potential for antiproliferation on MCF-7 breast and PC-3 prostate cancer cell lines which were in a dose-dependent manner.

Keywords: ***Garcinia mangostana*, antioxidation, antiproliferation, cytotoxicity**

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Introduction

Breast and prostate cancers are the most commonly diagnosed invasive cancers both in women and men and are considered to be 2 of the leading causes of death due to cancer in Thailand (Sriplung *et al.*, 2003; Kirttipornsakda *et al*., 2011). Breast and prostate cancers are difficult to treat due to several distinct classes of tumors that express different treatment responses (Middleton *et al*., 2000; Kirttipornsakda *et al*., 2011). Generally, surgery and radiation therapeutic treatment are not fully efficacious; therefore, chemotherapy needs to be used instead. However, there are many symptoms of chemotherapeutic side effects such as normal cell death, hair loss, vomit, and anemia (Haskell, 1985; Kirttipornsakda *et al*., 2011). Therefore, development of new therapeutic approaches against cancers, especially from natural products, still remains as one of the most important challenges.

In Thailand, there are many attractive plant species which potentially have therapeutic applications. Mangosteen (*Garcinia mangostana* Linn) has a long history of medicinal use in Southeast Asia, having been used as a traditional medicine to treat many illnesses and diseases such as abdominal pain, infected wounds, diarrhea, dysentery, haemorrhoids, arthritis, chronic ulcers, food allergies, suppuration, leucorrhoea, gonorrhea, and skin infections (Huang *et al.*, 2001; Leiseisen *et a*., 2004; Suksamran *et al*., 2003). Also, mangosteen powder is an ingredient in several commercial products including nutritional supplements, nutraceuticals, herbal cosmetics, and pharmaceutical products (Ji *et al*., 2007).

Mangosteen hull, the bio- waste, has been documented as being rich in xanthones (α-mangostin, β-mangostin, γ-mangostin, gartanin, garcinone B, garcinone E, 8-deoxygartanin, and 9-hydroxycalabaxanthone), tannins, and flavonoids (Obolskiy *et al*., 2009; Wexler, 2007). In the xanthone group, α- mangostin is the most studied due to its potential therapeutic properties (Walker, 2007; Li *et a*., 2013a). The remarkable pharmacological effects of xanthones include antifungal (Kaomongkolgit *et al*., 2009), antibacterial (Sakagami *et al*., 2005), antiviral (Chen *et al*., 1996), antioxidant (Jung *et al*., 2006), anti-inflammatory (Chen *et al*., 2008), anti-allergy (Nakatani *et al*., 2002), cardioprotective (Devi Sampath and Vijayaraghavan, 2007), neuroprotective (Weecharangsan *et al*., 2006), and anti-cancer (Moongkarndi *et al*., 2004a).

Recently, many research documents have supported the potential of α-mangostin in antiproliferation of human cancer cell lines such as HCT116 human colorectal cancer, SKBR3 human breast cancer, and PC12 human prostate cancer (Moongkarndi *et al*., 2004a; Sato *et al*., 2004; Aisha *et al*., 2012). Additionally, mangostin and xanthone derivatives from mangosteen hull could induce apoptosis via the extrinsic (involving the death receptor signaling) and intrinsic (involving mitochondrial cascades) pathways in leukemia cell lines HL60, K562, NB4, and U936, breast cancer cell SKBR3, and prostate cancer cell PC12 (Matsumoto *et al*., 2003; Moongkarndi *et al.*, 2004a; Sato *et al*., 2004).

Brine shrimp and mosquito larvae lethality assays are considered as a convenient inquiry for preliminary assessment of harmful effects. Both are generally used in medicinal plant research in order to estimate toxicity and biological actions, which some cases involved in pharmacological studies fulfill for various chemical compounds (Parra *et al*., 2001; Pisutthanan *et al*., 2004; Phoenok *et al*., 2015). Although mangosteen is a very favorite fruit for consumption, few people know about the toxicity of the hull.

The aim of this study is to investigate the phytochemicals of methanolic and ethanolic mangosteen hull extracts due to the different efficiency of the polarities of the solvents which can contribute different potentials of antioxidant activity. The methods for determining the antioxidant activities of the extracts were the use of DPPH$^{\bullet}$ scavenging and lipid peroxidation inhibition assays; the antiproliferative effects were tested on MCF-7 human breast cancer and PC-3 human prostate cancer cells. The effectiveness of the brine shrimp and mosquito larvae bioassays was evaluated to predict the

toxicity of the extracts. Information and knowledge from this study hopefully will be useful for pharmacological development and cancer therapy in the future.

Materials and Methods

Chemicals

Folin-Ciocalteu's phenol reagent, gallic acid (GA), catechin (CA), epigallocatechin-3-gallate (EGCG), iron (III) chloride hexahydrate ($FeCl_3 \bullet 6H_2O$), and 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$) were purchased from Sigma-Aldrich, St. Louis, MO, USA. Dulbecco's Modified Eagle's Medium: Nutrient Mixture F-12 (DMEM/F-12), Roswell Park Memorial Institute 1640 Medium (RPMI-1640), penicillin/streptomycin, fetal bovine serum (FBS), and resazurin were the GIBCO brand from Invitrogen, ThermoFisher Scientific Corp., Waltham, MA, USA. 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) was the Molecular Probes product from Invitrogen. All other chemicals and reagents were of analytical grade.

Mangosteen Collection and Hull Extraction

Mangosteen fruits were collected from a local farm in Makham, Chanthaburi Province, Thailand. The plant was taxonomically identified by the Department of Agriculture of Thailand, specimen voucher no. TBD-DOA-04-1-6156. The hulls were separated from the fruits, cleaned, dried, and ground to a powder that was extracted in 95% methanol or ethanol for 24 h by using a Soxhlet extraction apparatus. The extract was evaporated, lyophilized, and kept at -20ºC.

Quantification of Total Phenolic Compounds

Total phenolic compounds (TPC) were quantified by the Folin- Ciocalteu method (Singleton *et al*., 1999). One hundred microliters of sample were mixed with 2 ml of 2% sodium carbonate solution containing 100 µl Folin-Ciocalteu reagent (Folin-Ciocalteu's phenol reagent: absolute methanol, 1:1, v/v) and incubated for 45 min. The absorbance was measured at 760 nm. The TPC was expressed as micrograms of gallic acid equivalent (GAE) per micrograms/milligram of sample, using a standard curve generated with gallic acid.

$$Y = 0.0048x + 0.0615;\ R^2 = 0.9998 \qquad (1)$$

Quantification of Flavonoids Content

The flavonoids content (FC) was measured using a colorimetric method (Jia *et al*., 1999). One hundred and twenty- five microliters of sample were mixed with 0. 625 ml of dH_2O and 37.5 µl of 5% $NaNO_2$ for 10 min. Seventy-five microliters of 10% $AlCl_3$ were added and incubated for 5 min. After that, 0.25 ml of 1 M NaOH was added and the volume adjusted to 1.25 ml with dH_2O. The absorbance was measured at 510 nm. The FC was expressed as micrograms of catechin equivalent (CAE) per micrograms/milligram of sample, using a standard curve generated with catechin.

$$Y = 0.0027x + 0.0577;\ R^2 = 0.9999 \qquad (2)$$

Determination of Antioxidant Activity by Free Radical Scavenging Method

The antioxidant activity of the extract was investigated by $DPPH^{\bullet}$ (2,2-diphenyl-1-picrylhydrazyl) assay (Sanchez-Moreno *et al*., 2003). Fifty microliters samples at various concentrations were mixed with 1.95 ml DPPH reagent and incubated in the dark for 60 min. The absorbance was measured at 515 nm. The catechin (CA) and epigallocatechin-3-gallate (EGCG) were determined as a positive control. The scavenging activity was computed according to the below formula and expressed as the median effective concentration, EC_{50}.

$$Scavenging\ activity\,(\%) = \left[1 - \frac{(A_1 - A_2)}{A_0}\right] x\,100 \qquad (3)$$

where A_0 was the absorbance of the control, A_1 was the absorbance of the $DPPH^{\bullet}$ solution present in the sample, and A_2 was the absorbance without the $DPPH^{\bullet}$ solution.

Determination of Antioxidant Activity by Ferric Thiocyanate Method

The antioxidant activity of the extract was determined by ferric thiocyanate (FTC) assay (Ono *et al*., 2000; Huang *et al*., 2006).

A 1 ml sample was mixed with 1.5 ml of 2.51% linoleic acid solution and 2.5 ml of 0.05 M phosphate buffer (pH 7.0), and then incubated in the dark at 40ºC to accelerate the oxidative reaction. During incubation, a 0.05 µl solution was diluted with 4.85 ml of 75% ethanol and 0.05 µl of 30% ammonium thiocyanate, and incubated for 3 min. Fifty microliters of 0.02 M ferrous chloride in 3.5% hydrochloric acid were added and the absorbance was measured at 500 nm every 24 h until the maximum absorbance of the control. Catechin (CA) and epigallocatechin-3-gallate (EGCG) were determined as a positive control. Lipid peroxidation inhibition (LPI) was calculated using the below formula and analyzed for EC_{50} values.

$$\textit{Lipid peroxidation inhibition}\ (\%) = \left[1-\frac{(A_1-A_2)}{A_0}\right] x\,100 \quad (4)$$

where A_0 was the absorbance of the control, A_1 was the absorbance present in the sample, and A_2 was the absorbance without the potassium thiocyanate solution.

Cell Culture

Human breast cancer MCF-7 and human prostate cancer PC-3 cells were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). The MCF-7 cells were cultured in Dulbecco's Modified Eagle's Medium: Nutrient Mixture F-12 (DMEM/F-12), while the PC-3 cells were cultured in Roswell Park Memorial Institute 1640 Medium (RPMI-1640). Both mediums were supplemented with 10% FBS and 1% penicillin/streptomycin in a 5% CO_2 atmosphere at 37ºC.

Measurement of Cell Proliferation

Cell proliferation was assayed by 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT). This is a colorimetric assay based on the conversion of the yellow MTT solution to the purple formazan derivatives by mitochondrial succinate dehydrogenase in viable cells (Hansen *et al*., 1989). The MCF-7 and PC-3 cells at 10000 cells/well in 100 µl were plated onto a 96-well plate and incubated for 24 h. The cultured cells were treated with MHEs at various concentrations and continued to incubate for 24 h. The cultured medium was discarded and 100 µl of MTT solution in 5 µg/µl phosphate buffer saline at pH 7.4 was added and incubated for 4 h. Then, the MTT solution was discarded and 150 µl of absolute DMSO (dimethyl sulfoxide) was added. The plate was continually agitated until the formazan dissolved. The absorbance was measured at 570 nm with the reference wavelength at 630 nm. A decrease in the absorbance indicated a reduction of cell viability (Babu *et al*., 2002; Okonogi *et al*., 2007). Catechin (CA) and epigallocatechin-3-gallate (EGCG) were used as a standard control. The percentage of antiproliferation activity was calculated using the following formula and plotted against the concentrations of the samples. The median inhibitory concentration (IC_{50}) was derived from the best fit line obtained by linear regression analysis.

$$\textit{Antiproliferation activity}\ (\%) = \left[1-\frac{(A_1-A_2)}{(A_0-A_2)}\right]\times 100 \quad (5)$$

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

Measurement of Cytoprotective Activity

The resazurin method is a colorimetric assay based on the conversion of the purple resazurin solution to the red resorufin solution via reduction reactions of metabolically active cells. MCF-7 and PC-3 cells at 10000 cells/well were plated onto a 96-well plate and incubated for 24 h. The cultured cells were treated with MHEs at various concentrations and continually incubated for 24 h. The culture medium was discarded and a 100 µl resazurin solution in DMEM/F-12 without FBS was added and incubated for 2 h. The absorbance was measured at 570 nm with the reference wavelength at 600 nm. A decrease in absorbance indicated a reduction in cell viability (Goegan *et al*., 1995; Manasathien and Indrapichate, 2017). The percentage of cell death was calculated following Equation 5 and the IC_{50} value was analyzed.

Measurement of Brine Shrimp Lethality

Cytotoxicity was performed with the brine shrimp lethality assay (BSLA) as described by Solis *et al*. (1993). Brine shrimp (*Artemia salina*) eggs were purchased from a local fish shop. After larval hatching, they were reared in artificial seawater (120 g/l sea salt) under continuous light and a temperature at 25ºC for 24 h. Forty nauplii were transferred onto a 24-well plate containing MHEs solution at various concentrations for 24 h. The dead larvae were counted. The percentage of mortality was calculated according to the below formula and plotted against the concentrations of the samples. The median lethal concentration (LC_{50})was derived from the best fit line obtained by linear regression analysis.

$$Deaths\,(\%) = \left[1-\frac{(A_1-A_2)}{A_1}\right] x\,100 \qquad (6)$$

where A_1 was the live control (the medium without the sample), and A_2 was the deaths present in the samples.

Measurement of Mosquito Larvae Lethality

Cytotoxicity was performed with the mosquito larvae lethality assay (MLLA). Mosquito (*Aedes aegypti*) eggs were obtained from the Office of Disease Prevention and Control 5. After larval hatching, they were reared in tap water under continuous light and a temperature at 25ºC for 5 days. Twenty larvae were transferred onto a 6-well plate containing MHEs at various concentrations for 24 h. The dead larvae were counted. The percentage of mortality was calculated using Equation 6 and the LC_{50} value was analyzed.

Statistical Analysis

The experiments were repeated 4 times and all data were expressed as the mean ± standard deviation. Data were analyzed for multiple comparisons by 1-way ANOVA, using the least significant test to determine the level of significance at $P<0.01$. For single comparisons, the different significance of means was determined by the Student's T-test at the significant level of $P<0.01$.

Results and Discussion

Total Phenolic Compounds and Flavonoids Content

The total phenolic compounds (TPC), flavonoids content (FC), and antioxidant property are positively relative. In this study, the TPC and FC values of the mangosteen hull methanolic extract (MHE/m) were slightly higher than those of the ethanolic extract (MHE/e) at various concentrations, but there were no significant differences ($P>0.01$). The TPC value of the MHE/ m was more than that of the MHE/e (119.62±1.86>117.34±4.75 µg GAE/mg sample) (Table 1 and Figure 1), while the FC value was the same (52.87±1.05> 49.73±2.19 µg CAE/mg sample) (Table 1 and Figure 2).

Table 1. Total phenolic compounds and flavonoid contents of mangosteen hull extracts and their antioxidant capacities, assessed by DPPH and FTC methods

Sample	Total phenolics (µg GAE/mg)	Total flavonoids (µg CAE/mg)	DPPH EC_{50} (µg/ml)	LPI EC_{50} (µg/ml)
MHE/m	119.62±1.86	52.87±1.05	582.17±26.39^{c}	100.69±3.29^{c}
MHE/e	117.34±4.75	49.73±2.19	599.02±53.24^{c}	108.20±6.76^{c}
CA	-	-	101.34±5.91^{b}	6.51±0.21^{b}
EGCG	-	-	62.79±0.46^{a}	5.48±0.29^{a}

Values represent means ± S.D., n = 4.Values at each treatment group followed by different letters are significantly different ($P<0.01$). GAE, gallic acid equivalent; CAE, catechin equivalent; DPPH, 2,2-diphenylhydrazyl radical scavenging activity; LPI, lipid peroxidative inhibition; EC_{50}, median effective concentration; MHE/ m, mangosteen hull methanolic extract; MHE/e, mangosteen hull ethanolic extract; CA, catechin; epigallocatechin-3-gallate (EGCG)

The constituents of the phytochemicals are important for pointing out the antioxidant property and cytotoxicity of the plant. As for mangosteen hull, some of the literature has reported about various phenolic compounds such as flavonoids, tannin, and xanthones (Gopalakrishnan *et al*., 1997; Ji *et al*., 2007; Yu *et al*., 2007). The majority of phenolic compounds in mangosteen hull belongs to the xanthone family such as 8-desoxygartanin, and α-, β-, and γ-mangostins (Gopalakrishnan *et al*., 1997; Huang *et al*., 2001). In the current study, the TPC and FC of the MHE/m were slightly higher than those of the MHE/e, but did not significantly differ. That corresponds to the efficiency of the polarity of methanol over than that of ethanol. Methanol could sequester more phenolic, flavonoid, tannin, saponin, and alkaloid than ethanol (Widyawati *et al*., 2014). The α-mangostin, a naturally occurring xanthone, is water insoluble, and its difference in solubility in a variety of non-

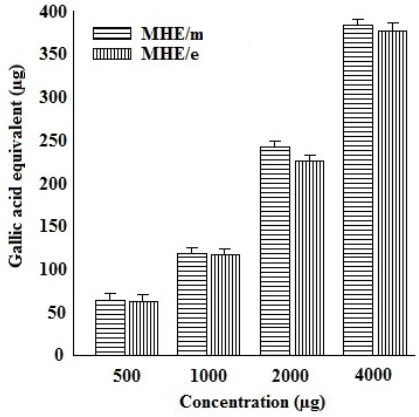

Figure 1. Total phenolic compounds of mangosteen hull extracts by methanol (MHE/m) and ethanol (MHE/e). Values represent means ± S.D., n = 4

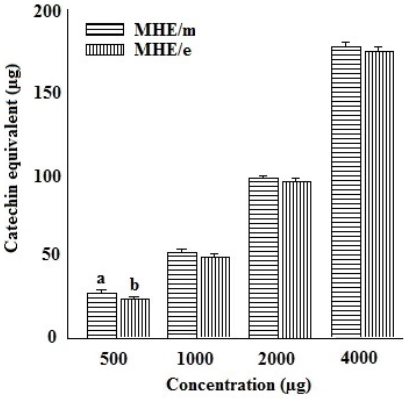

Figure 2. Flavonoids contents of mangosteen hull extracts by methanol (MHE/m) and ethanol (MHE/e). Values represent means ± S.D., n = 4. Values at each treatment group followed by different letters are significantly different (P<0.01)

polar solvents has been reported (Budavari, 1989). Manasathien and Khanema (2015) found that mangosteen hull extracted via 70% ethanol contained TPC higher than that extracted via water (119.95>84.40 μg GAE/mg). Consistently, absolute alcohol could isolate TPC and FC from mangosteen hull more than 50% ethanol could (Wang *et al*., 2012a). α-Mangostin and γ-mangostin are mainly chemical components in xanthones extract, approximately 81% and 16% , respectively (Chairungsrilerd *et al*., 1996). Also, Ngawhirunpat *et al*. (2010) reported that greater amounts of α-mangostin could be extracted with hexane (28.7% w/w) and methanol (15.5% w/w) than with water. However, the FC (6.8-8.7 g EE/100 g extract) and total tannin contents (36.4-37.7 g TAE/100 g extract) of mangosteen methanolic and hexane extracts were not significantly different (Ngawhirunpat *et al*., 2010). Therefore, it is obvious that xanthones in the MHEs are very important for antioxidant properties.

Antioxidant Properties

Antioxidant activity measurement by the DPPH method can be related to an ability of free radical scavenging and is represented via the EC_{50} value. By definition, a low EC_{50} value means a high ability of free radical scavenging. MHEs at 1000 μg/ml concentration could inhibit the DPPH radicals up to 78% and 77% (data not shown). The EC_{50} values of the MHE/ m and MHE/ e were 582.17±26.39 and 599.02±53.24 μg/ ml, respectively, and their antioxidant abilities were indicated to be lower than the positive controls such the CA and EGCG (101.34±5. 91 and 62.79±0.46 μg/ml, respectively) (P<0.01) (Table 1).

Also, the antioxidant activity can be measured by lipid peroxidation inhibition (LPI) via the FTC method and is expressed as an EC_{50} value. By definition, a low EC_{50} value means a high ability of the LPI. It was found that the LPI of the MHE/m and MHE/e were 100.69±3.29 and 108.29±6.76 μg/ml, respectively, which means a lower ability of the LPI than the CA and EGCG (6.51±0.21 and 5.48±0.29 μg/ml, respectively) (P<0.01) (Table 1). As a result, the MHE/m and MHE/e have a low ability of lipid peroxidation inhibition.

Generally, high TPC or FC values involve a high antioxidant capacity which can be found in many plant species (Leong and Shui, 2002), the same as with the mangosteen hull. In the current study, the MHEs contained amounts of phenolic compounds referring to antioxidant properties even though the study used different antioxidant methods (DPPH and FTC) based on different principles and natural antioxidants (CA and EGCG). This result corresponds to mangosteen hull methanolic (Kosem *et al*., 2007), 70% ethanolic, and water extracts (Manasathien and Khanema, 2015). However, the MHEs in this study possessed lower free radical scavenging activity than the CA and EGCG by approximately 6- and 9- fold, respectively. Moreover, the lipid peroxidation inhibitions of the MHEs were lower than those of the CA and EGCG by approximately 16- and 19- fold, respectively. The MHEs and xanthones isolated from the mangosteen hull demonstrated a pivotal role in antioxidant capacities via scavenging DPPH, ABTS, nitric oxide, and lipid radical in a dose-dependent manner (Yoshikawa *et al*., 1994; Leong and Shui, 2002; Kosem *et al*., 2007; Manasathien and Khanema, 2015). Also, α- and γ-mangostins exhibited antioxidant capacities that could be proved via the FTC method (Fan and Su, 1997). The study of α-mangostin documented that it could inhibit low density lipoproteins' oxidation (Williams *et al*., 1995; Mahabusarakam *et al*., 2000).

Antiproliferation Effect on MCF-7 Cells

Antiproliferation activity on human breast cancer MCF-7 cells can be investigated via the MTT and resazurin methods. MCF-7 cells were treated with MHEs at concentrations ranging from 0 to 600 μg/ml for 24 h, and then the percentage of cell viability and IC_{50} were calculated and are shown in Table 2. MHEs could decrease cell proliferation in a dose-dependent manner. In Table 2, IC_{50} values of MHE/ m and MHE/ e via the MTT method were 437.54±7.94 and 466.41±10.05 μg/ ml, respectively, which made their antiproliferative ability higher than the CA (2212.42±57.61

μg/ml) but lower than the EGCG (145.84±2.87 μg/ml). Consistently, IC_{50} values of MHE/m and MHE/e via the resazurin method were 462.42±11.94 and 473.58±10.55 μg/ml, respectively, which means a higher ability of antiproliferative activity than the CA (2911.48 ±209.53 μg/ml) but lower than the EGCG (154.27±6.07 μg/ml). As a result, the MHEs had more antiproliferative effects on the MCF-7 cells in a dose-dependent manner. The MHEs were more effective than the CA by approximately 5- and 6- fold via the MTT and resazurin methods, respectively (P<0.01), and were lower than the EGCG by about one-third.

For both the MTT and resazurin assays, the antiproliferative activity of the MCF-7 cells by the MHEs was significant in a dose-dependent manner. Also, some reports have documented that an antiproliferation property induced by xanthones was significantly active on the human umbilical vein endothelial (ECV304) cell line (Kosem *et al*., 2007), human epithelial type 2 (Hep-2) cell line (Chitra *et al*., 2010), and hepatocellular carcinoma (Ho *et al*., 2002). Mangosteen methanolic and ethanolic extracts showed an antiproliferative effect on the human breast cancer (SKBR3) cell line (Moongkarndi *et al*., 2004a; Moongkarndi *et al*., 2004b). Human melanoma cancer (SK-MEL-28) and squamous carcinoma (A- 431)cells induced apoptosis by MHE/e treatment (Wang *et a*., 2012b). α-Mangostin and γ-mangostin showed significant suppression of tumor growth and metastasis in a mouse model of mammary cancer (Doi *et al*., 2009). A-Mangostin induced cell cycle arrest, apoptosis, activation of caspase-3 and -9, release of cytochrome c, and loss of mitochondrial potential in BJMC3879 cells (Doi *et al*., 2009; Shibata *et al*., 2011). Manasathien and Khanema (2015) reported that 70% ethanolic mangosteen extract was a more potential inhibitor on MCF-7 cells' proliferation in a dose- and time-dependent manner. Moreover, 70% ethanolic extract could induce DNA fragmentation and nucleus condensation in apoptosis, but water extract could not. A-Mangostin and γ-mangostin could induce the mechanism of human leukemia (HL60) cell death and strongly inhibited human colon cancer (DLD-1) cell proliferation via cell cycle arrest and apoptotic pathway (Matsumoto *et al*. , 2003, 2004, 2005). Consistently, MHE and γ-mangostin affected MCF-7 cell cytotoxicity and induced antiproliferation via apoptosis pathway (Chitra *et al*., 2010). Moreover, α-mangostin suppressed

Table 2. Antiproliferative activities of mangosteen hull extracts on MCF-7 cells, assessed by MTT and resazurin assay

Sample	Conc. (μg/ml)	MTT		Resazurin	
		Antiproliferation (%)	IC_{50} (μg/ml)	Antiproliferation (%)	IC_{50} (μg/ml)
MHE/m	300	26.76±1.86^{d}	437.54±7.94^{b}	19.81±5.68^{d}	462.42±11.94^{b}
	400	41.94±3.00^{c}		32.22±3.62^{c}	
	500	56.12±3.11^{b}		53.55±1.07^{b}	
	600	84.37±1.27^{a}		83.29±1.33^{a}	
MHE/e	300	20.04±3.91^{d}	466.41±10.05^{c}	15.82±6.31^{d}	473.58±10.55^{b}
	400	31.72±5.78^{c}		31.85±4.68^{c}	
	500	50.69±1.90^{b}		48.26±2.69^{b}	
	600	82.37±3.13^{a}		82.97±1.93^{a}	
CA	1000	32.84±1.53^{b}	2212.42±57.61^{d}	6.70±3.18^{b}	2,911.48±209.53^{c}
	2000	43.33±3.23^{a}		37.17±2.35^{a}	
EGCG	100	16.72±0.48^{b}	145.84±2.87^{a}	13.00±1.21^{b}	154.27±6.07^{a}
	200	79.00±1.83^{a}		75.64±3.15^{a}	

Values represent means ± S.D., n = 4. Values at each treatment group followed by different letters are significantly different (P<0.01). MHE/m, mangosteen hull methanolic extract; MHE/e, mangosteen hull ethanolic extract; CA, catechin; epigallocatechin-3-gallate (EGCG). IC_{50}, median inhibitory concentration.

TPA-mediated matrix metalloproteinase-2/9 (MMP-2 and MMP-9) expressions through the extracellular signal-regulated kinase (ERK) 1/2 pathway in MCF-7 cells (Lee *et al*., 2010). According to the literature, it could be seen that MHEs had antiproliferative effects on MCF-7 cells.

Antiproliferation Effect on PC-3 Cells

Human prostate cancer PC-3 cells were used to find the rates of cell proliferation and cell viability via the MTT and resazurin methods. After 0 to 300 µg/ml MHEs were treated for 24 h, cytoprotective activity of the PC-3 cells was investigated and calculated for the percentage of cell viability (Table 3). As a result, the MHEs were partially potent against PC-3 cell proliferation in a dose-dependent manner. In Table 3, the proliferation potency and IC_{50} values of MHE/m and MHE/e via the MTT method were 233.33±1.63 and 239.0±2.06 µg/ml, respectively, which showed that their antiproliferative ability was more than the CA (1027.57±83.37 µg/ml) but lower than the EGCG (190.05±18.15 µg/ml). Similarly, the IC_{50} values of the MHE/m and MHE/e via the resazurin method were 295.92 ± 1.27 and 305.56 ± 5.93 µg/ml, respectively, which means that their antiproliferative ability was more than the CA (1265.46 ± 126.09 µg/ml) but lower than the EGCG (208.78±18.92 µg/ml). For both the MTT and resazurin methods, the effectiveness of the MHEs was greater than the CA by approximately 4.3- and 4.2-fold, respectively (P<0.01), but lower than the EGCG by approximately 1.2- and 1.4-fold (P<0.01).

Xanthones and α-mangostin in mangosteen hull extracts have anticancer properties via cell cycle arrest and apoptosis initiation, which the pathway can express as a suppression of cancer cell proliferation and a metastasis through the inhibition of anti- apoptotic molecules (Shan *et al*., 2011). A-Mangostin is responsible for anticancer activity in colorectal, lung, breast, and prostate cancer (Gutierrez-Orozco and Failla, 2013; Ibrahim *et al*., 2016). Sittisombut *et al*. (2012) found that α- mangostin from mangosteen hull could induce high antiproliferative activity against human colon cancer cells (HCT116 and HT29 at 80.0% and 75.5% inhibition, respectively) and human prostate cancer cells (PC-3 at 78.5% inhibition). The IC_{50} values of α-mangostin on prostate cancer cell lines (LNCaP, 22Rv1, DU145, and PC-3) were 5.9, 6.9, 22.5, and 12.7 µM, respectively

Table 3. Antiproliferative activities of mangosteen hull extracts on PC-3 cells, assessed by MTT and resazurin assay

Sample	Conc. (µg/ml)	MTT		Resazurin	
		Antiproliferation (%)	IC_{50} (µg/ml)	Antiproliferation (%)	IC_{50} (µg/ml)
MHE/m	150	12.90±2.60^{d}	233.33±1.63^{b}	14.81±3.79^{d}	295.92±1.27^{b}
	200	30.18±2.11^{c}		29.94±1.72^{c}	
	250	58.17±2.27^{b}		36.54±1.64^{b}	
	300	82.85±2.45^{a}		51.91±0.86^{a}	
MHE/e	150	8.83±3.34^{d}	239.20±2.06^{c}	12.34±4.41^{d}	305.56±5.93^{c}
	200	26.69±2.14^{c}		27.51±0.88^{c}	
	250	55.25±2.15^{b}		35.07±1.25^{b}	
	300	81.22±3.01^{a}		49.56±1.15^{a}	
CA	500	26.05±9.01^{b}	1027.57±83.37^{d}	26.47±3.04^{b}	1265.46±126.09^{d}
	1000	48.06±1.18^{a}		37.86±4.43^{a}	
EGCG	100	23.69±5.16^{b}	190.05±18.15^{a}	20.47±3.35^{b}	208.78±18.92^{a}
	200	54.09±4.35^{a}		49.44±4.07^{a}	

Values represent means ± S.D., n = 4. Values at each treatment group followed by different letters are significantly different (P<0.01). MHE/m, mangosteen hull methanolic extract; MHE/e, mangosteen hull ethanolic extract; CA, catechin; epigallocatechin-3-gallate (EGCG). IC_{50}, median inhibitory concentration.

(Johnson *et al*., 2012). Consistently, xanthones in MHE could induce a cytotoxic effect on PC-3 cells at the IC_{50} value of 10 µg/ml and the viability assay displayed a time-dose dependent growth inhibitory activity (Kirttipornsakda *et al*., 2011). Mangosteen extract could induce apoptosis, reduce viability and proliferation of prostate cancer cells, and suppress tumor growth in a xenograft tumor model (Li *et al*., 2013b). α-Mangostin could promote cell cycle arrest in PC3 cells by targeting on cyclin-dependent kinase 4 (CDK4), which was determined by a cell free kinase assay (Johnson *et al*., 2012). Hung *et al*. (2009) and Wang *et al*. (2012b) reported that xanthones in mangosteen exhibited anti-metastatic activity on PC-3 cells through inhibition of c-Jun NH_2-terminal kinase (JNK) 1/2, matrix metalloproteinase-2/9 (MMP-2 and MMP-9), nucleus factor-κB (NF-κB), activator protein 1 (AP-1), and urokinase-plasminogen (u-PA). Xanthone could induce apoptosis by DNA fragmentation and nucleus condensation. Moreover, xanthone could induce the expression of caspase-3 and caspase-8 indicating the apoptosis of PC-3 cells via the death receptor pathway (Kirttipornsakda *et al*., 2011). These results indicate that MHEs have a partial antiproliferative effect on PC-3 cells.

Cytotoxic Effect

The cytotoxicity of the MHEs was performed via the brine shrimp lethality assay (BSLA) and mosquito larvae lethality assay (MLLA). After 24 h of treatment, the surviving brine shrimps and mosquito larvae were counted and calculated for the percentages of death and LC_{50} values. In this study, the percentage of death for both animals increased in a dose-dependent manner (Table 4). The LC_{50} values of the MHE/m and MHE/e via the MLLA (277.55±14.98 and 355.01±32.37 µg/ml, respectively) were significantly different, but the values via the BSLA (153.88±3.37 and 161.75±5.09 µg/ml, respectively) were not (Table 4).

The BSLA and MLLA are useful for preliminary assessment of cytotoxicity. In the evaluation of plant toxicity via the BSLA and MLLA, the LC_{50} values below 1000 µg/ml are regarded as bioactive (Meyer *et al*., 1982; Parra *et al*., 2001). In this study, the LC_{50} values of MHEs in the BSLA and MLLA ranged from 153.88 to 161.75 and from 277.55 to 355.01 µg/ml, respectively (Table 4). Therefore, the MHEs were more toxic to brine shrimps and mosquito larvae after 24 h. Similarly, by the BLSA, the LC_{50} values of *Derris malaccensis, Euphorbia marginata*, *Antidesma*

Table 4. Cytotoxicity of mangosteen hull extracts assessed by brine shrimp and mosquito larvae lethality

Sample	Conc. (µg/ml)	Brine shrimp		Mosquito larvae	
		Death (%)	LC_{50} (µg/ml)	Death (%)	LC_{50} (µg/ml)
MHE/m	50	6.88±2.39^{d}	153.88±3.37	0.00±0.00^{d}	277.55±14.98^{a}
	100	28.75±5.20^{c}		5.00±4.08^{c}	
	200	71.25±3.23^{b}		26.25±4.79^{b}	
	300	100.00±0.00^{a}		56.25±4.79^{a}	
MHE/e	50	5.00±2.04^{d}	161.75±5.09	0.00±0.00^{c}	355.01±32.37^{b}
	100	26.88±6.88^{c}		0.00±0.00^{c}	
	200	68.75±5.95^{b}		16.25±4.79^{b}	
	300	95.00±2.04^{a}		37.50±6.45^{a}	

Values represent means ± S.D., n = 4. Values at each treatment group followed by different letters are significantly different (P<0.01). MHE/m, mangosteen hull methanolic extract; MHE/e, mangosteen hull ethanolic extract. LC_{50}, median lethal concentration

nigricans, *E. lathyris*, and *Bridelia retusa* were 0.33, 162, 286, 333 and 363 µg/ml, respectively, which were all lower than 1000 µg/ml (Plangklang and Athibai, 2014; Meyer *et al*., 1982). Sala and Manasathien (2016) reported that after 24 h treatment with 70% ethanolic and methanolic mangosteen hull extracts the LC_{50} values of mosquito larvae were 271.21 and 266.49 µg/ml. α-Mangostin was strongly cytotoxic in human keratinocyte cells (LC_{50} 0.94 µg/ml), while the subordinates were hexane (LC_{50} 30 µg/ml) and methanol (LC_{50} 72 µg/ml). However, water extract did not show an adverse effect on viable cells (Ngawhirunpat *et al*., 2010). Therefore, cytotoxicity depends upon a quantity of α- mangostin extracted from the mangosteen hull, which directly involves a kind of solvent.

Conclusions

Although mangosteen hull is a bio-waste, it is still of benefit in the study of phytochemical property. In this study, the findings found that MHEs possessed low antioxidant capacities when compared with standard antioxidants such catechin and epigallocatechin-30-gallate. MHEs were more powerful in antiproliferation against cancerous MCF-7 and PC-3 cells in a dose-dependent manner when compared with the standard antioxidants. MHEs also expressed more toxicity on brine shrimps and mosquito larvae. The toxicological risk assessment and pharmacological data from this study could be beneficial for clinically therapeutic development. It is suggested that more study of MHEs' toxicity should be done on normal cell lines, animals, and humans.

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