JOURNAL OF NATURAL REMEDIES
DOI: 10.18311/jnr/2016/842

Cytotoxic Activity of Mangosteen (*Garcinia mangostana* L.) Peel Extract and α-Mangostin toward Leukemia Cell Lines (HL-60 and K-562)

Arina Novilla[1], Dedi S. Djamhuri[1], Nurul Fauziah[2], Maesaroh Maesaroh[2], Balqis Balqis[2] and Wahyu Widowati[3]*

[1]School of Health Sciences Jenderal Achmad Yani, Cimahi, Indonesia
[2]Biomedical and Biomolecular Research Centre, Aretha Medika Utama, Bandung, Indonesia
[3]Faculty of Medicine, Maranatha Christian University, Bandung, Indonesia; wahyu_w60@yahoo.com

Abstract

Fruit of Mangosteen (*Garcinia mangostana* L.) is well-known in Indonesia and other Southeast Asian countries. Studies have shown that extract of the pericarp of mangosteen contained mostly of xanthones exhibit many biological activities, especially as an antitumor. This study aimed to investigate the cytotoxic activity and selectivity of Mangosteen Peel Extract (MPE) and α-mangostin against two leukemia cell lines (HL-60 and K-562) and the normal lymphocyte cells from two different donors. The cytotoxic activity was performed using 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay. Imatinib and Isotretinoin were used as a positive control to the K-562 and HL-60 cells, respectively. The MPE and α-mangostin revealed higher mortality toward leukemia cell lines rather than toward lymphocyte cells, with more than 80% of HL-60 and K-562 cells died at 6.25 and 25 µg/ml, respectively. MPE was more toxic and selective against K-562 with IC50 of 2.79 µg/ml and SI of 8.27, while α-mangostin was more toxic and selective against HL-60 with IC50 of 1.12 µg/ml and SI of 22.34. MPE and α-mangostin showed potent sensitivity and selectivity to leukemia cells, hence these are considered as promising sources for future leukemia treatment.

Keywords: Mangosteen Peel Extract, α-mangostin, acute myeloid leukemia (AML), chronic myeloid leukemia (CML), selectivity index

1. Introduction

Cancer has been the second cause of death after cardiovascular diseases in the world. There were around 8.2 million of people died because of cancer and about 14.1 million of new cancer cases were detected in 2012[1]. Particularly in Indonesia, there was about 0.1% of total people suffering from cancer with 74.6% of mortality rate in male and 63.3% in female[2]. Lung or bronchus cancer, prostate cancer in men, breast cancer in the female, and colon or rectum cancer reveal as the most deadly cancer worldwide. However, rare cancer such as leukemia is also a cause of death because of its destructive nature[3]. According to the GLOBACAN, the percentage of leukemia was 2.5% of total new cases, contributing to 3.2% of total deaths by cancer in 2012[1] which was mostly suffered by children[4].

**Author for correspondence*
Email: wahyu_w60@yahoo.com

Characterized by high numbers of abnormal white blood cells, leukemia has been grouped into lymphoblastic and myeloid leukemia, namely Chronic Lymphocytic Leukemia (CLL) or Acute Lymphocytic Leukemia (ALL) and Chronic Myelogenous Leukemia (CML) or acute Myelogenous Leukemia (AML). Leukemia can be treated using interferon-α (IFN-α), Stem Cell Transplantation (SCT), radiotherapy and chemotherapy. Imatinib, a chemotherapy agent of tyrosine-kinase inhibitor, has been used as the first-line treatment for CML. It induces apoptosis and inhibition of proliferation by inhibiting the phosphorylation and binding process of Bcr-Abl oncoprotein[5]. Another chemotherapy agent for leukemia is retinoic acid, vitamin A derivative such as All-Trans Retinoic Acid (ATRA) and isotretinoin. They are used for several cancer treatment and remission including for Acute Promyelocytic Leukemia (APL), a subtype of AML by inducing cell apoptosis[6]. However, the usage of imatinib and retinoic acid as well as the other chemotherapy agents can generate side effects by damaging the normal cells and lead to the development of the multidrug resistance cancer[7]. Therefore, the finding of the safe, selective, and sensitive chemicals that have anticancer property is still needed.

Mangosteen (*Garcinia mangostana* L.) has been used as a traditional medicine to treat diseases related to immune and gastrointestinal for decades. Its extract contains several active compounds, including terpenes, anthocyanins, tannins, phenols, and xanthones[8]. Xanthones have been shown to have several pharmacological properties, including as an antitumor[9]. The most abundant and the most studied xanthones are α-mangostin, β-mangostin, γ-mangostin, garcinone E, and gartanin, and those xanthones possess great biological activities such as anti-allergy, anti-inflammatory, antioxidant, anti-tumor, antibacterial, antifungal and antiviral activities, neuroprotective, cardioprotective, and immunomodulation[10–12]. Prior studies revealed that α-mangostin were the most abundance xanthone derivative from the aqueous, acetone and methanol extract of mangosteen[9,13]. Therefore, this study aimed to investigate the cytotoxic activity and selectivity of Mangosteen Peel Extract (MPE) and α-mangostin against two leukemia cell lines (HL-60 and K-562) and the normal lymphocyte cell from two different donors. The 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium MTS) assay was used to investigate the cytotoxic effect of the MPE, α-mangostin, imatinib, and isotretinoin. Those data are used to reveal the IC_{50} and Selectivity Index (SI) of the MPE and three other compounds to the HL-60 and K-562 cell lines. The *in vitro* result demonstrates that MPE and α-mangostin have a potential to use against leukemia by showing the higher cytotoxic effect and selective index to leukemia rather than the imatinib and isotretinoin.

2. Materials and Methods

2.1 Mangosteen Peel Extract (MPE) Preparation

The peels of *Garcinia mangostana* L. from Cisalak-Subang, West Java, Indonesia were determined by the herbarium staff of Biology Department, School of Life Sciences and Technology, Bandung Institute of Technology, Bandung, West Java, Indonesia. Around 350 g of chopped and dried-mangosteen peels were extracted using steady-state extraction (maceration) method. The chopped and dried peels were immersed in ethanol 70% for 24 hours and filtrated. The filtration was repeated until the filtrate became colorless. The filtrate was evaporated with a rotary evaporator at 40°C and the ethanolic extract of mangosteen peel was collected and stored in a freezer with the temperature of -20°C[12,13].

2.2 Leukimia Cell Lines Cultivation

Two different leukemia cell lines, human acute promyelocytic (HL-60) and human chronic myelogenous (K-562) were obtained from Stem Cells and Cancer Institute (SCI) Jakarta, Indonesia. Cell lines were cultured in supplemented Iscove's Modified Dulbecco's Medium (IMDM) (Biowest, France), 2% Penicillin-Streptomycin (Biowest, France), 10% Fetal Bovine Serum (FBS) (Biowest, France) and were incubated at 37°C in a humidified incubator with 5% CO_2. After 24 hours of incubation, the number of viable cells was counted using a haemocytometer with tryphan blue staining to obtain a sufficient cell number for the cytotoxic assay.

2.3 Lymphocyte Isolation and Cultivation

Lymphocyte isolation from two healthy donors was approved by Ethic Commission Number 232/KEP/I/2016 of Maranatha Christian University-Immanuel Hospital Bandung, Indonesia. The healthy donors have signed the informed consent. After taken from donors, to separate

the lymphocyte from other leukocytes histopaque (Sigma-Aldrich, U.S.A.) was added into the whole blood. Whole blood then was centrifuged at 1,800 rpm for 5 minutes to obtain the blood buffy coat. The blood buffy coat was added with 1x Phosphate Buffer Saline (PBS) (Biowest, France) and was centrifuged at 1,800 rpm for 10 minutes in 4ºC. Then, the pellet was added with 1x PBS and was centrifuged at 1,500 rpm for 10 minutes in 4ºC. The final pellet contained leukocytes was maintained in Roswell Park Memorial Institute Medium (RPMI) (Biowest, France) and supplemented with 1% non-essential amino acids, 1% 1-glutamine, 100 U/ml penicillin, 10mg/mL streptomycin and 10% heat-inactivated fetal calf serum (Biowest, France) then incubated at 37ºC in humidified air containing 5% CO_2[14].

2.4 Cytotoxic Assay

Approximately 100 µL of lymphocyte, HL-60 and K-562 cell suspension contained 10^5 cells/ml were seeded in each well of 96-well plate. Following that, 100 µL of Isotretinoin (Roche, Switzerland) in different levels (100, 85, 70, 55, 40, and 25 µM) were added into the wells of lymphocyte and HL-60. Imatinib (Glivec, Novartis) in different levels (100, 50, 25, 12.5, 6.25, and 3.125 µM) were added into the wells of lymphocyte and K-562. α-mangostin (Cengdu Biopurify Phytochemical, China) (100, 50, 25, 12.5, 6.25, and 3.125 µM) and Mangosteen Peel Extract (MPE) (100, 50, 25, 12.5, 6.25, and 3.125 µg/mL) were added into the wells of lymphocytes, HL-60, and K-562. Cells then incubated at 37°C with 5% of CO_2 for 24 hours. Then, 20 µL of 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxyme-thoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) (Promega, U.S.A.) was added into each well and plate was incubated at 5% CO_2, 37ºC for 3 hours. Finally, the absorbance used to calculate the percentage of cell mortality (Equation 1) was measured at 490 nm with Multiskan GO (Thermo Scientific, U.S.A). Wells of each cell that not supplemented with the compounds were presented as the negative control.

$$\text{Cell Mortality}\,(\%) = \frac{\left(A_{\text{untreated cell}} - A_{\text{treated cell}}\right)}{A_{\text{untreated cell}}} x\ 100\% \quad (1)$$

$A_{\text{treated cell}}$: Absorbance of cells treated with either MPE, α-mangostin, imatinib, or isotretinoin;

$A_{\text{untreated cell}}$: Absorbance of untreated cells

2.5 Selectivity Analysis

The Selectivity Index (SI) describes the selectivity of the MPE, α-mangostin, imatinib, and isotretinoin toward the leukemic cell lines. The index was calculated from IC_{50} ratio of the lymphocytes and the cancerous cell line (HL-60 or K-562) (Equation 2)[15]. The compound with SI higher than 3 revealed a high selective compound against the HL-60 or K-562, and the opposite was a less selective compound.

$$\text{Selectivity Index (SI)} = \frac{IC_{50}\ \text{of normal cell}}{IC_{50}\ \text{of cancer cell}} \quad (2)$$

IC_{50} of normal cell: The value of median inhibitory concentration (IC_{50}) of lymphocyte cells;

IC_{50} of cancer cell: The value of median inhibitory concentration (IC_{50}) of cancer cells

2.6 Statistical Analysis

All treatment in this study was done independently and conducted in triplicate. SPSS Statistic 17.0 for Windows was used to calculate the IC_{50} of the compounds and the significant differences using probit analysis and one-way ANOVA, respectively. The mortality and viability data were presented as mean ± Standard Deviation (SD).

3. Results

3.1 Cytotoxic Effect of Each Compound toward HL-60, K-562, and Leukocyte

Table 1 to 4 show the cytotoxic effect of imatinib, isotretinoin, α-mangosteen and MPE presented as the percentage mortality of HL-60, K-562, and leukocyte. Isotretinoin and α-mangostin started at the concentration of 25 µM had more than 50% mortality to HL-60 and K-562, respectively. Imatinib and α-mangostin started at 12.5 µM showed more than 50% mortality to K-562 and HL-60, respectively (Table 1-3). Those of MPE started at 3.125 µg/ml and at 6.25 µg/ml revealed more than 50% mortality to HL-60 and K-562, respectively (Table 4). Furthermore, Figure 1 shows HL-60 and K-562 cell lines have lower density in a higher concentration of MPE and α-mangostin treatments.

Table 1: The percentage mortality (%) of Lymphocyte and K-562 treated with Imatinib in six different concentrations

Cells	Concentration (μM)					
	100	50	25	12.5	6.25	3.125
Lymphocyte	74.67 ± 11.09^{b}	10.71 ± 5.82^{a}	6.09 ± 6.48^{a}	5.67 ± 6.57^{a}	-5.81 ± 1.92^{a}	-22.88 ± 10.86^{a}
K-562	94.64 ± 0.11^{f}	76.89 ± 1.01^{e}	63.43 ± 0.74^{d}	55.95 ± 0.99^{c}	51.95 ± 0.9^{b}	47.5 ± 0.8^{a}

Data is presented as mean ± standard deviation, different superscript letters in each row (Lymphocyte, K-562) showed a significant difference at p<0.05 (Tukey HSD post hoc test) among the concentrations.

Table 2: The percentage mortality (%) of Lymphocyte and HL-60 treated with Isotretinoin in six different concentrations

Cells	Concentration (μM)					
	100	85	70	55	40	25
Lymphocyte	91.74±12.07^{b}	60.39±17.70^{b}	54.93±34.16^{b}	39.4±14.56^{ab}	15.05±4.31^{ab}	-38.14±11.43^{a}
HL-60	99.05±1.11^{e}	95.41±2.19^{de}	83.48±1.02^{cd}	80.53±2.48^{bc}	69.52±4.66^{b}	51.73±2.86^{a}

Data is presented as mean ± standard deviation, different superscript letters in each row (Lymphocyte, HL-60) showed a significant difference at p < 0.05 (Tukey HSD post hoc test) among the concentrations.

Table 3: The percentage mortality (%) of Lymphocyte, HL-60, and K-562 treated with α-Mangosteen in six different concentrations

Cells	Concentration (μM)					
	100	50	25	12.5	6.25	3.125
Lymphocyte	67.53±25.83^{aA}	49.9±1.88^{aA}	33.8±26.82^{aA}	29.32±7.07^{aA}	22.6±37.28^{aA}	19.38±1.11^{aB}
HL-60	95.72±0.43^{dA}	94.55±0.07^{dB}	94.51±0.65^{dA}	75.1±0.27^{cB}	-29.44±0.5^{bA}	-45.36±7.4^{aA}
K-562	96.2±0.11^{dA}	94.65±0.3^{dB}	91.29±0.27^{dA}	16.31±0.65^{cA}	-21.72±3.08^{bA}	-68.47±8.35^{aA}

Data is presented as mean ± standard deviation, different lowercase of superscipt letters in each row (Lymphocyte, K-562, HL-60) showed a significant difference at p<0.05 (Tukey HSD post hoc test) among the concentrations, different uppercase of superscript letters in each column (100, 50, 25, 12.5, 6.25, 3.125) showed a significant difference at p<0.05 (Tukey HSD post hoc test) among the cells.

Table 4: The percentage mortality (%) of Lymphocyte, HL-60, and K-562 treated with MPE in six different concentrations

Cells	Concentration (μg/ml)					
	100	50	25	12.5	6.25	3.125
Lymphocyte	94.96±12.46^{cA}	55.91±8.82^{bA}	44.86±1.15^{abA}	42.2±1.46^{abA}	37.3±5.4^{abA}	16.17±3.71^{aA}
HL-60	106.7±1.85^{bcA}	100.97±0.67^{bB}	106.8± 0.52b^{cC}	109.1±1.7^{cC}	101.65±1.36^{bC}	89.7±1.11^{aC}
K-562	98.08±0.83^{dA}	95.06±2.95^{cdB}	93.14±4.21^{cdB}	86.24±0.76^{cB}	73.16±0.23^{bB}	46.23±0.65^{aB}

Data is presented as mean ± standard deviation, different lowercase of superscript letters in each row (Lymphocyte, K-562, HL-60) showed a significant difference at $p < 0.05$ (Tukey HSD post hoc test) among the concentrations, different uppercase of superscript letters in each column (100, 50, 25, 12.5, 6.25, 3.125) showed a significant difference at $p < 0.05$ (Tukey HSD post hoc test) among the cells.

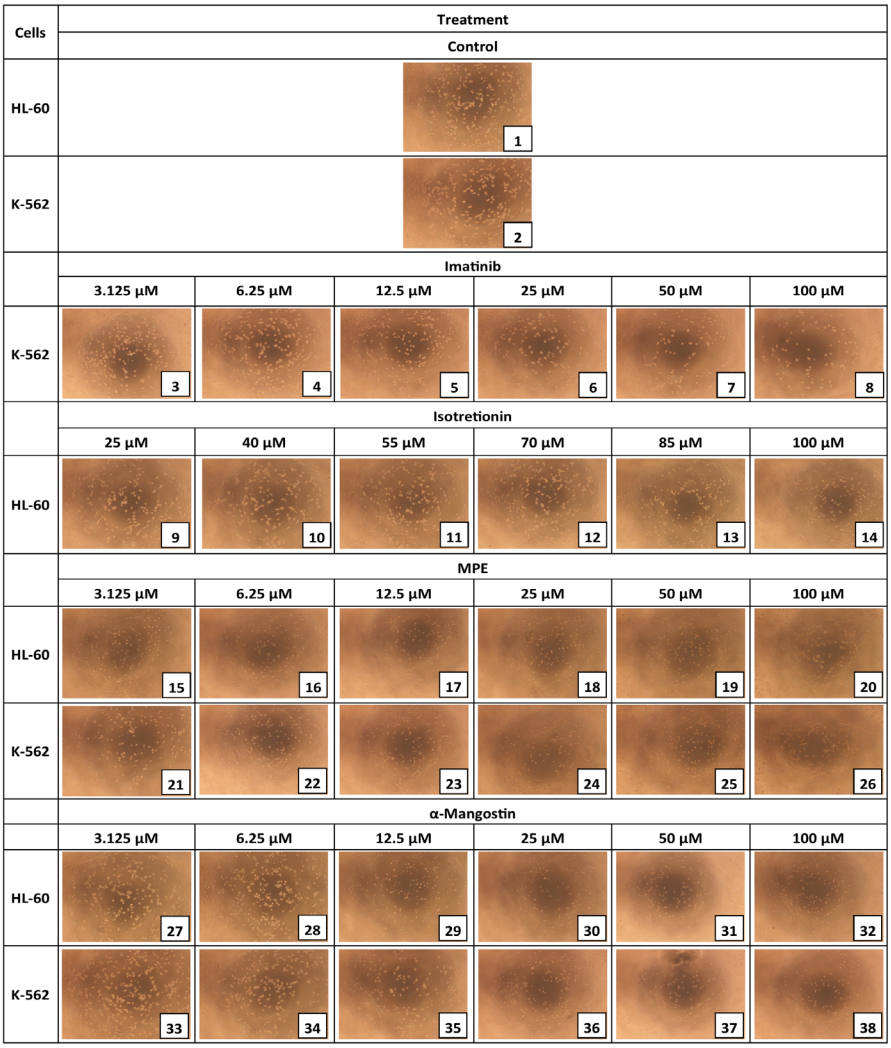

Fig. 1. Morphological appearance of HL-60 and K-562 cells with no treatment (1,2), treated with Imatinib (3-8), Isotretinoin (9-14), MPE (15-26), and α-mangostin (27-38). Scale bar: 200 µm. Control (untreated) cells showed higher density than treated cells. The density of HL-60 and K-562 were low in higher MPE and α-mangostin concentrations.

3.2 Sensitivity and Selectivity of Each Compound toward HL-60, K-562, and Leukocyte

Further analysis of percentage mortality of the compounds using probit regression revealed that the IC_{50} of imatinib, isotretinoin, MPE, and α-mangostin were high to normal lymphocyte and the IC_{50} MPE and α-mangostin were comparably low to the leukemia cell lines (Figure 2). MPE possessed the lowest IC_{50} (2.79 µg/ml) toward K-562 whereas α-mangostin possessed the lowest IC_{50} (1.12 µg/ml) to HL-60 (Table 5). The result of selectivity index showed that isotretinoin, a recent chemotherapy agent used to treat leukemia was less selective than MPE and α-mangostin toward HL-60, whilst imatinib was more selective toward K-562 than MPE and α-mangostin. Nevertheless, MPE and α-mangostin showed high selectivity toward both of HL-60 and K-562, with selectivity index was more than 3 (Table 5).

Table 5: The cytotoxicity and selectivity of MPE, α-mangostin, imatinib and isotretinoin against HL-60, K-562, and leukocyte

Compounds	Cell Line				
	HL-60		K-562		Lymphocyte
	IC_{50} (µg/ml)	SI[a]	IC_{50} (µg/ml)	SI[a]	IC_{50} (µg/ml)
MPE[b]	1.16	19.83	2.79	8.27	23.08
α-mangostin	1.12	22.34	7.21	3.47	25.02
Isotretinoin	7.66	2.86	-	-	21.93
Imatinib	-	-	2.84	12.07	34.35

a. Selectivity Index, calculated based on the ratio of IC50 lymphocyte and the respective leukemic cell line.

b. Mangosteen peel extract.

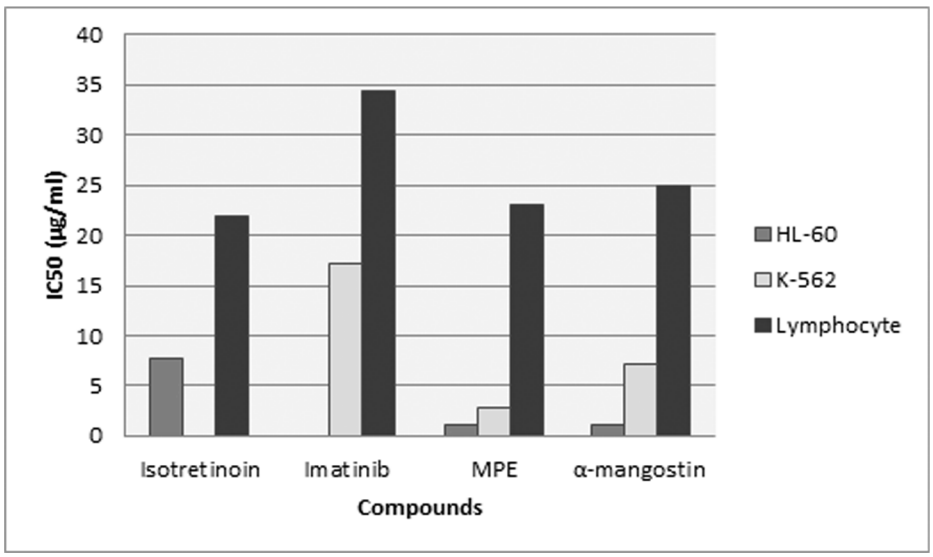

Fig. 2. IC_{50} values of isotretinoin, imatinib, Mangosteen Peel Extract (MPE) and α-mangostin toward leukemia cell lines (HL-60 and K-562) and normal lymphocyte.

4. Discussion

The activities of xanthones and its derivates to inhibit certain molecular target in cancer progression are related to the tricyclic scaffold and its position[8]. As an anti-cancer, xanthones arrest cell cycle, induce apoptosis, and differentiation but inhibit the tumor cell proliferation, adhesion, invasion, and metastasis[16–18]. Xanthones were also reported to prevent initiation stage of cancer by inducing Quinine Reductase (QR) and inhibiting cytochrome P450 (CYP) activity[9,19].

In this study, imatinib, isotretinoin, MPE, and α-mangostin showed a cytotoxic effect on the HL-60, K-562, and lymphocyte cells in a concentration-dependent manner. Microscopic images showed the cell density gradually decreased in the higher concentration of each treatment (Figure 1). This result was supported by the percentage of mortality data that showed the cell mortality increased in higher concentration of each treatment. Based on the Table 5, the IC_{50} value of MPE and α-mangostin toward HL-60 and K-562 cell lines was lower than the IC_{50} value toward lymphocyte, with the selectivity index higher than 3. These findings suggested that MPE and α-mangosteen were safe against the normal lymphocyte, and possessed a high selectivity and sensitivity toward HL-60 and K-562[8,15]. These results were consistent with Matsumoto *et al.*[22] study that showed the antiproliferative activity of xanthones in mangosteen pericarp against human leukemia[20]. The α-mangostin mediates mitochondrial apoptotic pathway in human promyelocytic leukemia (HL-60) by activating

the caspase-3 and caspase-9[21]. Together with β-mangostin, γ-mangostin, and methoxy-β-mangostin, these compounds arrest the cell cycle via expression of cyclin proteins in the human colon cancer cells (DLD-1)[11]. Furthermore, other anti-tumor activities of mangostins in several cancers have also been reported, including the inhibition of TCF/β-catenin transcription in colon cancer cells and inhibition of cell growth signaling pathways in chondrosarcoma[22]. These findings suggest that xanthones work by various pathways to cancer cells.

In contrast, isotretinoin was either less selective or less sensitive toward the HL-60 cells compared with MPE and α-mangostin. Cancer has been known to be able to develop resistance towards chemotherapy. The resistance of leukemic cell lines to retinoic acid derivate might occur in molecular level by affecting several proteins functions and the mutations of the RARα receptor in APL can block the initiation of differentiation by retinoic acid[23]. On the contrary, Imatinib shows good sensitivity and selectivity toward K-562 human Chronic Myeloid Leukemia (CML) cells. CML is characterized by the presence of a Bcr-Abl fusion gene, which is caused by a reciprocal translocation of chromosomes 9 and 22[24]. The cytotoxic activity of imatinib was supported by other study, demonstrated that imatinib was able to inhibit Bcr-Abl kinase activity led to inactivate of survival pathways and induce long-term activation of caspases that responsible for the degradation and inactivation of Bcr-Abl tyrosine kinase as well as apoptosis of the K562 cells[25].

5. Conclusion

Take together, our data suggest that MPE and α-mangostin possessed potent sensitivity and selectivity against leukemia. Both of them revealed higher selectivity and sensitivity than isotretinoin toward HL-60 cell line, while MPE also show high sensitivity and selectivity toward K-562 cell line, showing its great potential for pharmaceutical application. We suggest that MPE can be produced as a safe, efficient and low cost of an alternative remedy to fight leukemia. Therefore, the further study of mangosteen peel extract in molecular and *in vivo* study must be conducted.

6. Acknowledgements

We gratefully acknowledge the financial support from Research Grant of Hibah Bersaing 2016 by Ministry of Research, Technology and Higher Education of the Republic of Indonesia and Biomedical and Biomolecular Research Centre Aretha Medika Utama, Bandung, Indonesia for research grant, research method and facilities support. We are also thankful to I Dewa Gde Sathya Deva from Biomedical and Biomolecular Research Centre Aretha Medika Utama, Bandung, Indonesia for his valuable assistance.

7. References

1. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, et al. Cancer incident and mortality worldwide: Source, methods and major patterns in GLOBOCAN 2012. Int J Cancer. 2014; 136:349–86.
2. McDonald M, Hertz R, Lowcnthal SW. Pfizer facts: The burden of cancer in Asia USA: Pfizer Medical Division; 2008.
3. Modak H, Kulkarin SS, Kadakol GS, Hiremath SV, Patil BR, Hallikeri U, et al. Prevalence and risk of leukemia in the multi-ethnic population of North Karnataka. Asian Pac J Cancer Prev. 2011; 12:671–5.
4. Belson M, Kingsley B, Holmes A. Risk factors for accute leukemia in children: A review. Environ Health Persp. 2007; 115(1):138–45.
5. Henkes M, van der Kuip H, Aulitzky WE. Therapeutic options for chronic myeloid leukemia: focus on imatinib (Glivec®, GleevecTM). Ther Clin Risk Manag. 2008; 4(1):163–87.
6. Niles RM. Recent advances in the use of Vitamin A (Retinoids) in the prevention and treatment of cancer. Nutr. 2000; 16:1084–90.
7. Gottesman MM, Fojo T, Bates ES. Multidrug resistance in cancer. Nat Rev Cancer. 2002; 2:48–58.
8. Shan T, Ma Q, Guo K, Liu J, Li W, Wang F, et al. Xanthones from mangosteen extracts as natural chemopreventive agents: potential anticancer drugs. Curr Mol Med. 2011; 11(8):666–77.
9. Foti RS, Pearson JT, Rock DA, Wahlstrom JL, Wienkers LC. In vitro inhibition of multiple cytochrome P40 isoforms by xanthone derivatives from mangosteen extract. Drug Metab Dispos. 2009; 37(9):1848–55.
10. Yang J, Liu RH, Halim L. Antioxidant and antiproliferative activities of common edible nut seeds. Lwt-Food Sci Technol. 2009; 42(1):1–8.
11. Aisha AFA, Abu-Salah KM, Ismall Z, Abdul-Majid AMS. In vitro and in vivo anti-colon cancer effects of Garcina

mangostana xanthones extract. BMC Compl Alternative Med. 2012; 12(104):1–10.

12. Darsono L, Hidayat M, Maesaroh M, Fauziah N, Widowati W. Ex vivo study of *Garcinia mangostana* L. (Mangosteen) peel extract and xanthones as anti-adipogenesis in HepG2 cell model. Int J Med Res Health Sci. 2015; 4(3):566–71.
13. Widowati W, Darsono L, Suherman J, Yelliantty Y, Maesaroh M. High Performance Liquid Chromatography (HPLC) analysis, antioxidant, antiaggregation of mangosteen peel extract (*Garcinia mangostana* L.). Int J Biosci Biochem Bioinfo. 2014; 4(6):458–66.
14. Boyum A. Isolation of mononuclear cells and granulocytes from human blood. Isolation of monuclear cells by one centrifugation, and of granulocytes by combining centrifugation and sedimentation at 1 g. Scand J Clin Lab Invest Suppl 1968; 97:77–89.
15. Mahavorasirikul W, Viyanant V, Chaijaroenkul W, Itharat A, Na-Bangchang K. Cytotoxic activity of Thai medicinal plants against human cholangiocarcinoma, laryngeal and hepatocarcinoma cells in vitro. BMC Complement Altern Med. 2010; 10(55):1–8.
16. Akao Y, Nakagawa Y, Iinuma M, Nozawa Y. Anti-cancer effects of xanthones from pericarps of mangosteen. Int J Mol Sci. 2008; 9:355–70.
17. Pedraza-Chaverri J, Cardenas-Rodriguez N, Orozco-Ibarra M, Perez-Rojas JM. Medicinal properties of mangosteen (*Garcinia mangostana*). Food Chem Toxicol. 2008; 46:3227–39.
18. Hung SH, Shen KH, Wu CH, Liu CL, Shih YW. Alpha-mangostin suppresses PC-3 human prostate carcinoma cell metastasis by inhibiting matrix metalloproteinase-2/9 and urokinase-plasminogen expression through the JNK signaling pathway. J Agric Food Chem. 2009; 57(4):1291–8.
19. Chin YW, Jung HA, Chai H, Keller WJ, Kinghorn AD. Xanthones with quinone reductase-inducing activity from the fruits of *Garcinia mangostana* (Mangosteen). Phytochemistry. 2008; 69(3):754–8.
20. Matsumoto K, Akao Y, Kobayashi E, Ohguchi K, Ito T, Tanaka T, et al. Induction of apoptosis by xanthones from mangosteen in human leukemia cell lines. J Nat Prod. 2003; 66:1124–7.
21. Matsumoto K, Akao Y, Yi H, Ohguchi K, Ito T, Tanaka T, et al. Preferential target is mitochondria in alpha-mangostin-induced apoptosis in human leukemia HL60 cells. Bioorg Med Chem. 2004; 12(22):5799–806.
22. Krajarng A, Nakamura Y, Suksamram S, Watanapokasin R. Preferential target is mitochondria in alpha-mangostin-induced apoptosis in human leukemia HL60 cells. J Agric Food Chem. 2011; 59:5746–54.
23. Niles RM. Recent advances in the use of vitamin A (Retinoids) in the prevention and treatment of cancer. Nutr. 2000; 16:1084–90.
24. Deininger M, Buchdunger E, Druker BJ. The development of imatinib as a therapeutic agent for chronic myeloid leukemia. Blood. 2005; 105(7):2640–53.
25. Jacquel A, Herrant M, Legros L, Belhacene N, Luciano F, Pages G, et al. Imatinib induces mitochondria-dependent apoptosis of the Bcr-Abl-positive K562 cell line and its differentiation toward the erythroid lineage. FASEB J. 2003; 17(14):2160–2.