

Ethanollic Extract of Mangosteen (*Garcinia mangostana*) Peel Inhibits T47D and Hela Cells Line Proliferation Via Nf- κ B Pathway Inhibition

Erlina Rivanti, Annishfia Lailatur Rohmah, Herwandhani Putri, Prisnu Tirtanirmala, Dyaningtyas Dewi Putri Pamungkas*

Cancer Chemoprevention Research Center, Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta
 Jalan Sekip Utara 555281 (Phone. 0274 6492662 Fax. 543120) <http://ccrc.farmasi.ugm.ac.id>

Abstract

Effective and selective chemotherapeutic and chemopreventive agent is needed to cure breast and cervical cancers. One of the potential natural material is mangosteen peel (*Garcinia mangostana*). In this study, we observed cytotoxic effect of ethanolic extract of mangosteen peel (EMP) on HeLa cells line and T47D cells line. The cytotoxic effect was determined using MTT assay. EMP showed cytotoxic effect on T47D cells and HeLa cells with IC₅₀ values of 2.07 μ g/ml and 10.58 μ g/ml respectively. Molecular docking simulation was done to predict the molecular mechanism of active compound in mangosteen peel extract, α -mangostin, in NF κ B pathway which is one of the potential pathway to induce cytotoxicity on T47D and HeLa cells. Docking was done using PLANTS software and the binding score between α -mangostin and proteasom is -78,12, whereas the binding score between α -mangostin and IKK is -86.84. These results showed the possibility mechanism of mangostin peel extract containing α -mangostin inhibits IKK activation in NF κ B pathway. Based on this study, we conclude that mangosteen peel extract is potential to be developed as chemopreventive agent toward cervical and breast cancers.

Keywords: Mangosteen peel (*Garcinia mangostana*), cytotoxic, T47D cells, HeLa cells, NF κ B

INTRODUCTION

Cancer is a group of disease characterized by uncontrolled growth and spread of abnormal cells that result in death if the spread is uncontrolled (ACS, 2011). Women have a greater potential of developing this disease, including cervical cancer and breast cancer. Based on data collected by WHO in 2006, incidence of breast cancer are 502,000 and that is one of the leading causes of cancer death in the worldwide after lung cancer, liver, and colon. On the other hand, cervical cancer also has high incidence in the world even ranked first as a cause of death in women in Indonesia (Lewis and Merle, 2004; Aziz, 2009).

So far, many researches reported that some natural compounds exhibited as negative regulator of oncogenes and positive regulator of tumor suppressor gene that has potentially to be used as a cytotoxic agent in cancer, such as mangosteen (*Garcinia mangostana*). Previous

studies, as research conducted by Matsumoto (2005), proved that mangosteen peel extract inhibits the growth of DLD-1 cancer cells (human colorectal cancer cell model) at low doses of 5-20 μ M. This is most likely related to the presence of xanthon as major compounds in mangosteen peel, namely α -mangostin (Chairungrilerd *et al.*, 1996). Biological activity of α -mangostin has been known to have antiproliferative and apoptosis effects in liver cancer (Ho *et al.*, 2002), leukemia (Matsumoto, 2003), and colorectal cancer (Nakagawa *et al.*, 2007). In addition, α -mangostin also inhibit metastasis in PC-3 cancer cells. (Hung *et al.*, 2009). Therefore, further exploration is needed to know the potentially of mangosteen as an effective chemopreventive agent in breast and cervical cancers in women.

*Corresponding author e-mail: dyaningtyas.dewi@gmail.com

This study evaluated cytotoxic activity of ethanolic extract of mangosteen peel on T47D cells and HeLa cells. Cytotoxic activity was determined by MTT assay. In addition, molecular docking was conducted against several proteins associated with cells proliferation, such as proteasome and IKK. The results of this study are expected to be the basis for the development of mangosteen (*Garcinia mangostana*) as chemopreventive on cervical cancer and breast cancer.

MATERIALS AND METHODS

Plant Collection and Identification

Powder of mangosteen peel (*Garcinia mangostana*) were obtained from Purworejo, Central Java in June 2011 and was identified. Powder was extracted by maceration method for 5 days with 70% ethanol (E.Merck, Darmstadt, Germany).

Cells Culture

T47D cells and HeLa cells are collection of Cancer Chemoprevention Research Center (CCRC) Faculty of Pharmacy Universitas Gadjah Mada obtained from Prof. Tatsuo Takeya (Nara Institute of Science and Technology (NAIST), Japan).

Reagents

DMEM (Dulbecco's Modified Eagle Medium) for T47D cells and HeLa cells were obtained from Gibco. Culture medium contains fetal bovine serum (FBS) 10% (v/v) (FBS qualified, Gibco, Invitrogen TM USA) and 10,000 units/ml penicillin-10,000 µg/ml streptomycin (Gibco). Cells were harvested from the culture dish/flask using 0.25% Trypsin EDTA (Gibco, Invitrogen, Canada) to detached the cells. Reagent MTT {3-(4,5-dimethylthiazol-2-yl)2,5-diphenyl tetrazolium bromide} with stock 5 mg/ml in phosphate buffered saline (working reagent was prepared by diluting the stock reagent 10 times with DMEM medium), the sample solvent DMSO (Dimethyl sulfoxide) (Sigma Aldrich Chemie GmBH, Steinheim, Germany), stopper reagent contains sodium dodecyl sulfate (SDS, Merck -Schuchardt, Dr.Th.Schuchardt & Co., D-85 662 Hobenbrunn, Germany) 10% in 0.01 N HCl (Merck, Darmstadt, Germany), and Phosphate Buffer Saline wash solution (PBS) pH 7.4 were made with dissolving 0.2 g of KCl (for molecular biology, ≥ 99.0% HPLC pure (Sigma

Aldrich Chemie GmBH, Steinheim, Germany); 8 g NaCl (for molecular biology, ≥ 98% pure HPLC (Sigma Aldrich Chemie GmBH, Steinheim, Germany), 1.15 g Na₂HPO₄ (for molecular biology, ≥ 98.5% HPLC pure (Sigma Aldrich Chemie GmBH, Steinheim, Germany), 0.2 g of KH₂PO₄ (for molecular biology, ≥ 98% HPLC pure (Sigma Aldrich Chemie GmBH, Steinheim, Germany) in 1 liter of distilled water.

Cytotoxic Assay

Cytotoxic assay was done using MTT [3-4, 5-dimethylthiazol-2-yl)-2,5 difeniltetrazolium bromide] (Mosmann, 1983). Cells in 80% confluent were harvested and counted, then diluted with complete culture medium. The cells then were transferred into a 96-well plate with a total of 5 x 10³ cells/well followed by overnight incubation. After that, the sample was added to the wells at various concentrations with co-solvent DMSO and incubated at 37°C in a 5% CO₂ incubator for 24 hours. At the ends of incubation, 100 µL MTT reagent with concentration 0.5 mg/ml in DMEM was added into each well. The plate then incubated at 37°C for 3 hours until formazan was formed. If formazan was formed, stopper SDS 10% in 0.01N HCl was added. Plate then was wrapped with paper or aluminium foil and incubated in dark condition overnight. Absorbance was determined with an ELISA reader at λ 595 nm.

Molecular Docking Using Software YASARA, MarvinSketch, and PLANTS

Protein structure of the proteasome (PDB ID: 2F16) and IKK (PDB ID: 3RZF) as the docking target were downloaded through the website <http://www.pdb.org/pdb/home/home.do>. File structure of the target proteins that has been obtained then processed using YASARA software to set environmental conditions in accordance with the human physiological condition so that the simulation of complex formation between the target proteins really approaching original condition in the body. The structure of α-mangostin was drawn using MarvinSketch software. Protein and ligand then were processed using PLANTS to obtain the docking score. The ligand interactions with the protein was visualized using MOE.

Statistical Analysis

Data that collected from ELISA Reader are absorbances of each well and converted in percent of cell viability. Percent of cell viability were calculated using the equation:

$$\% \text{ viability cell} = \frac{A - B}{C - B} \times 100\%$$

A = Absorbance cells treatment
 B = Absorbance control medium
 C = Absorbance control cells

IC₅₀ concentration were calculated by the concentration that causes 50% inhibition of cell growth (Doyle and Griffiths, 2000). Calculation of IC₅₀ values were done using the linear

regression of concentration versus cells viability.

RESULTS AND DISCUSSION

Cytotoxic Effect of EMP on T47D Cells

MTT assay was used to evaluate the cell viability. The results suggested that EMP at the concentration of 5 µg/ml (Fig. 1B) altered the morphological appearance of T47D cells compared to control cells (Fig. 1A). Higher concentration of EMP (10 µg/ml) caused lower cell viability (Fig. 1C).

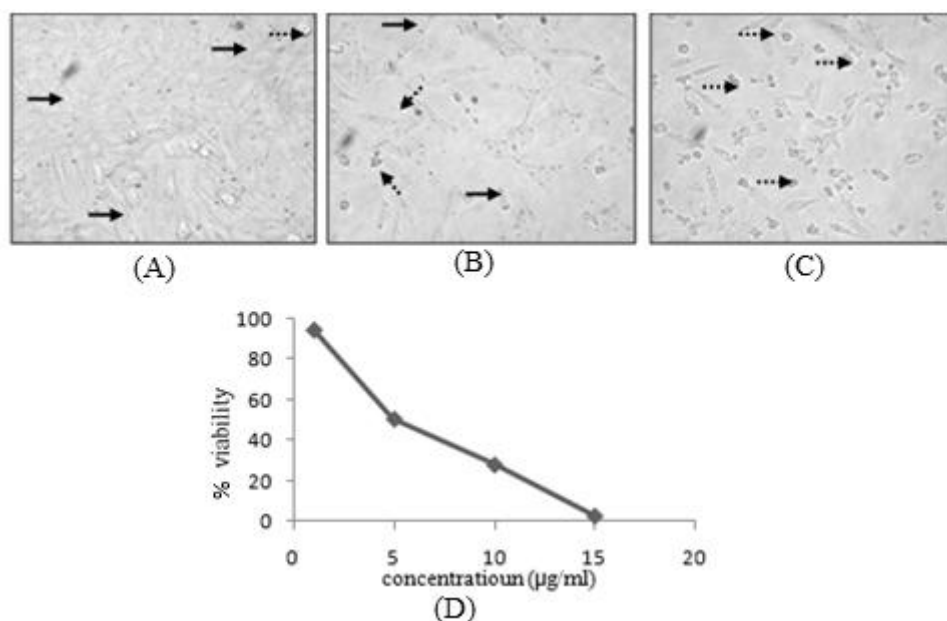


Figure 1. Cytotoxic effect of EMP on T47D cells. T47D cells with density were treated with EMP (1-15 µg/mL) and incubated for 24 hours. Cells viability were obtained from the conversion of absorbance values of formazan that formed by MTT treatment as described in the research procedure. Morphological appearance of control cells (A), cells treated with EMP 5 µg/mL (B) and 10 µg/mL (C). EMP treatment reduced cell viability with dose-dependent manner (D). Observation of cell morphology performed at the 24 hours using an inverted microscope with 100x magnification. Description: —→ normal T47D cells,→ cells that have undergone morphological changes.

Based on morphological appearance and cells viability, EMP with concentration of 1 µg/mL, 5 µg/mL, 10 µg/mL, and 15 µg/mL showed a linear correlation between concentration extract with toxicity effects. The IC₅₀ value of EMP on T47D cells was 2.07 µg/ml (p<0.05).

Cytotoxic Effect of EMP on HeLa Cells

On HeLa cells, the cytotoxic effects of EMP also showed dose-dependent phenomenon (Fig. 2). EMP performed cytotoxic effect to HeLa cells with IC₅₀ value of 10.58 µg/mL.

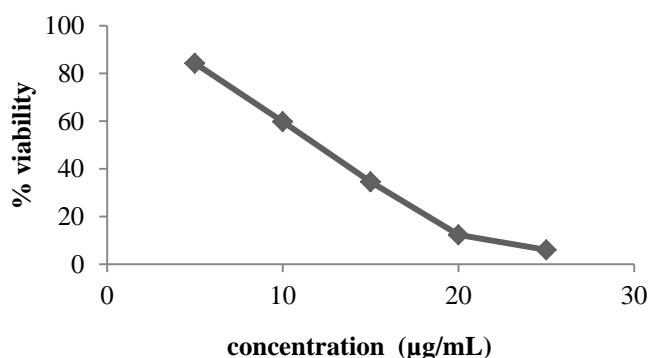


Figure 2. Cytotoxic effect of EMP on HeLa cells. HeLa cells were treated with EMP (1-25 µg/mL) and incubated for 24 hours. Cells viability were obtained from the conversion of absorbance values of formazan that formed by MTT treatment as described in the methods.

Molecular Docking between α -mangostin with Proteasome and IKK

Docking is a computational technique to predict whether a molecule (ligand) can bind to the receptor. Affinity was measured based on the energy stability of the system. The most stable interaction has low or negative free energy.

In this study, PLANTS software used to perform docking simulations between α -

mangostin with several proteins. Before the docking simulations, target proteins was downloaded through www.pdb.org and prepared using the YASARA software. Ligands (compounds) which will dock to target proteins were prepared using MarvinSketch software. The results of the docking simulations between α -mangostin with the proteasome and IKK are presented in Table I and II.

Table I. Docking score between α -mangostin and native ligand with proteasom

Ligand	Score
α -mangostin	-76.017
BO2 (bortezomib)	-103.736

RMSD = 5.3527 °A

Table II. Docking score between α -mangostin and native ligand with IKK

Ligand	Score
α -mangostin	-85.7415
C ₂₃ H ₂₄ C ₁ N ₅ O ₂	-95.1468

RMSD = 8.4398 °A

Alfa-mangostin showed higher docking score (lower affinity) compared to proteasome's native ligand (bortezomib). Bortezomib is a highly potent proteasome inhibitor and has been used widely in cancer therapy. On the contrary, α -mangostin was more potent to bind with IKK than the native ligand. Moreover, α -mangostin gave lower docking score with IKK rather than proteasome. In visualization of ligand interaction, the number of amino acids interact to change the conformational of structure ligand on proteosom was less than IKK. Moreover, the number of amino acids' proteasome that altered by ligand is less than IKK (Table III). This result suggested that α -mangostin preferably interfere IKK activity than proteasome in NF κ B pathway.

Active compounds in mangosteen acting as anticancer agent is α -mangostin (Chairungsri *et al.*, 1996). In this study, we evaluated the cytotoxic activity of ethanolic extract of mangosteen peel (EMP) on T47D and HeLa cells. This extract performed cytotoxic activity in both cells with IC₅₀ values less than 100 µg/mL. Ueda *et al.* (2002) suggested that plant extract with IC₅₀ less than 100 µg/mL is potential to be developed as anti-cancer agent.

Both T47D and HeLa cells have survival mechanism against death signals. In HeLa cells, p53 level was decreased due to protein E6 expressed by HPV. The E6 protein bind to p53 and degrade p53 (Lane, 1992).

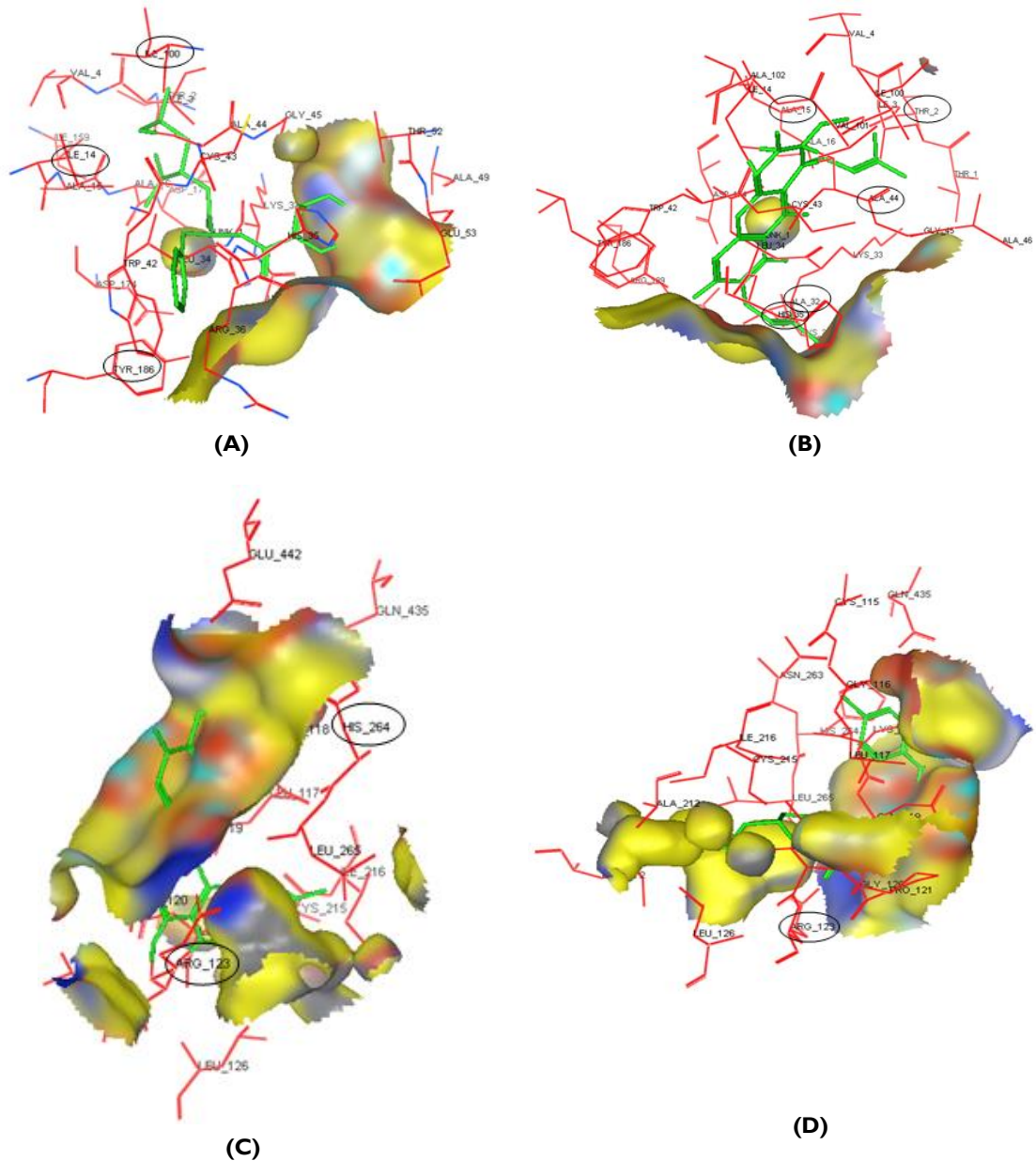


Figure 3. Visualization of Ligand Interactions between α -mangostin with residue of amino acids on Proteasome and IKK. Interaction between ligand and protein were visualized using MOE. The ligand is showed with green chain. Ligand interactions between α -mangostin with residue of amino acids on proteasome (A), BO2 (native ligand) with residue of amino acids on proteasome (B), α -mangostin with residue of amino acids on IKK (C), and $C_{23}H_{24}C_1N_5O_2$ (native ligand) with residue of amino acids on IKK (D). These images showed residues of amino acids that has interaction with ligand and could change the ligand's conformational.

Table III. Residues of Amino Acids interacts with ligand

Protein Target	Ligand	Amino Acids	Interaction	Distance
Proteasome (2F16)	α -mangostin	Ileum 100	H-donor	2.77
		Tyrosine 186	H-donor	2.61
		Ileum 14	Ionic	1.62
		Alanine 44	ionic	1.47
		Threonine 2	H-donor	2.15
		Alanine 15	H-donor	2.78
	BO2 (bortezomib)	Alanine 32	H-donor	1.71
		Threonine 52	H-donor	2.80
		Lysine 33	H-acceptor	2.54
		Histidine 35	Ionic	1.70
		Alanine 44	Ionic	1.72
		IKK (3RZF)	α -mangostin	Arginine 123
Histidine 264	Ionic			1.70
$C_{23}H_{24}C_1N_5O_2$	Arginine 123		H-acceptor	2.98

Darma *et al.* (2010) reported that in addition to p53 deficiency, survival mechanisms of HeLa cells is also associated with the high expression of Bcl-2, which involving NF κ B pathway. Whereas in T47D breast cancer cells, overexpression of Akt occurs and then activates the transcription factor NF κ B that act as upstream for various antiapoptosis proteins, such as Bcl-2. Therefore, NF κ B pathway become a potential target in this study that use both of cell models.

NF κ B pathway is one of the important pathways in the development of cancer cells due to its role as a transcription factor for some proteins, including antiapoptosis proteins like Bcl-2, Bcl-x1, and BFL1 (Gilmore, 2006). For its activation, NF κ B requires several steps: phosphorylation of I κ B by IKK, and I κ B degradation by proteasome (Karin and Ben, 2000). Excessive activation of NF κ B causes resistance in some cancer cells (Wang *et al.*, 1999). Abrogation of p53, as occurs in cervical cancer cells, increase NF κ B activation through TNF-alfa pathway (Weisz *et al.*, 2007). Moreover, Akt, which is overexpressed in T47D cells, can also induce activation of NF κ B by activating IKK first (Meng *et al.*, 2002).

Both in T47D and HeLa cells, NF κ B pathway plays a central role for cell survival. There are two strategies to inhibit this pathway: the inhibition of IKK activity or inhibition of I κ B degradation by the proteasome. Based on molecular docking, the data showed that α -mangostin exhibited better affinity with IKK rather than proteasome. However, it still needs to be proven further by looking IKK activity at the molecular level *in*

vitro. In addition, the expression of anti-apoptotic protein such as Bcl-2 should be reviewed to ensure that EMP can trigger apoptosis by decreasing the expression of antiapoptosis genes. Further study also needs to be done is to combine EMP with chemotherapeutic agent, leading to its potency to be a co-chemotherapeutic application

ACKNOWLEDGEMENT

We acknowledge Cancer Chemoprevention Research Center (CCRC) which has funded this research.

REFERENCES

- American Cancer Society, 2011, Cancer Facts and Figures 2011, available from <http://www.cancer.org/research/cancerfactsfigures/cancerfactsfigures/cancer-facts-figures-2011>, accessed December 2011.
- Aziz, M.F., 2009, Gynecological cancer in Indonesia, *J Gynecol Oncol*, **20**(1), 8–10.
- Chairungsrilerd, N., Furukawa, K., Ohta, T., Nozoe, S. and Ohizumi, Y., 1996, Pharmacological properties of alpha-mangostin, a novel histamine H1 receptor antagonist. *Eur J Pharmacol*, **314**(3), 351-356.
- Darma, A.P., Ashari, R.A., Nugroho, P.A., Monikawati, A., Fauzi, I.A., Hermawan, A. and Meiyanto, E., 2011, Aktivitas Sitotoksik Ekstrak Etanolik Herba Ciplukan (*Physalis angulata* L.) Pada Sel

- Kanker Leher Rahim HeLa Melalui Modulasi Ekspresi Protein p53, *Farmasains Jurnal Farmasi dan Ilmu Kesehatan*, **1**(2), 103-112.
- Doyle, A. and Griffiths, J.B., 2000, *Cell and Tissue Culture for Medical Research*. New York: John Wiley and Sons Ltd.
- Gilmore T.D., 2006, Introduction to NF κ B: players, pathways, perspectives, *Oncogene*, **25**, 6680-6684
- Ho, C.K., Huang, Y.L. and Chen, C.C., 2002, Garcinone E, a xanthone derivative, has potent cytotoxic effect against hepatocellular carcinoma cell lines, *Planta Med.*, **68**(11), 975-979.
- Huang, S., Wu, Y., Yu, H., Zhang, P., Zhang, X., Ying, L. and Zhao, H., 2005, Inhibition of Bcl-2 expression by a novel tumor-specific RNA interference system increases chemosensitivity to 5-fluorouracil in HeLa cells, *Acta Pharmacol Sin.*, **27**, 242-248.
- Hung S.H., Shen K.H., Wu C.H., Liu C.L. and Shih Y.W., 2009, 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.*, **57**(4), 1291-1298.
- Karin, M. and Ben-Neriah, Y., 2000, Phosphorylation meets ubiquitination: the control of NF- κ B activity, *Annu. Rev. Immunol.*, **18**, 621-63.
- Lane, D.P., 1992, p53, Guardian of The Genome, *Nature*, **358**, 15-16.
- Lewis, M.J., 2004, A Situational Analysis of Cervical Cancer Latin America & the Caribbean. Pan American Health Organization. Washington, D.C.
- Matsumoto, K., Akao, Y., Kobayashi, E., Ohguchi, L., Ito, T., Tanaka, T., linuma, M. and Nozawa, Y., 2003, Induction of Apoptosis by Xanthenes from Mangosteen in Human Leukemia Cell Lines, *J Nat Prod.*, **66**(8), 1124-1127
- Matsumoto K., Akao Y., Ohguchi K., Ito T., Tanaka T., linuma M. and Nozawa Y., 2005, Xanthenes induce cell-cycle arrest and apoptosis in human colon cancer DLD-1 cells, *Bioorg. Med. Chem.*, **13**, 6064-6069.
- Meng, F., Liu, L., Chin, P.C., and D'Mello, S.R., 2002, Akt is a Downstream Target of NF κ B, *Biol.Chem.*, **277**, 29674-29680.
- Mosmann, T., 1983, Rapid Colorimetric Assay for Cellular Growth & Survival: Application to Proliferation & Cytotoxicity Assays, *J Immunol Methods.*, **65**, 65-59.
- Nakagawa, Y., linuma, M., Naoe, T., Nozawa, Y. and Akao, Y., 2007, Characterized mechanism of α -mangostin-induced cell death: Caspase-independent apoptosis with release of endonuclease-G from mitochondria and increased miR-143 expression in human colorectal cancer DLD-1 cells, *Bioorg Med Chem*, **16**, 5620-5628.
- Ueda, J.Y., Tezuka, Y., Banskota, A.H., Tran, Q.L., Tran, Q.K., Harimaya, Y., Saiki, I. and Kadota, S., 2002, Antiproliferative Activity of Vietnamese Medicinal Plants, *Biol. Pharm. Bull.*, **25**(6), 753-760.
- Wang, S., Kotamraju, S., Konorev, E., Kalivendi, S., Joseph, J. and Kalyanamaran, B., 2002, Activation of nuclear factor- κ B during doxorubicin-induced apoptosis in endothelial cells and myocytes is pro-apoptotic: the role of hydrogen peroxide, *Biochem. J.*, **367**, 729-740.
- Weisz, L., Oren, M. and Rotter, V., 2007, Transcription Regulation by Mutant p53, *Oncogene*, **26**, 2202-2211.