EVALUATIONS OF FREE RADICAL SCAVENGING AND ANTIPROLIFERATIVE ACTIVITY OF FRUIT PEEL EXTRACT

R.Salfarina[1,3*], S. Nurul Nadiah[1], Z. Nur Atikah[1], M.N Nurain Zulaika[2]

[1] *Faculty of Pharmacy, Universiti Teknologi MARA*
[2]*Faculty of Health Sciences, Universiti Teknologi MARA*
[3]*Integrative Pharmacogenomics Institute (iPROMISE), Universiti Teknologi MARA Puncak Alam Campus, 42300 Bandar Puncak Alam, Selangor Darul Ehsan*
**e-mail : salfarina2892@puncakalam.uitm.edu.my*

Abstract

Fruit peels are known as waste of restaurant, market, and byproducts of food and beverages industries. They are usually discarded but are currently recognized as important sources for valuable phytochemicals, thus the exploration of its biological activity is encouraged. The fruit peel extract in this study was a combination of dried peels derived from mango; papaya and honeydew. The dried fruit peels were combined and macerated with 95% ethanol at a 50^{o}C using water bath. This study was intended to investigate the scavenging effect of fruit peel extract that was high in phenolic content against DPPH free radical and nitric oxide (NO). The MTT assay was carried out to evaluate the anti-proliferative activity of fruit peel extract on A549 and HepG2 cells which were lung cancer cells and liver cancer cells, respectively. The antiproliferation of the extract towards normal cell lines WRL68; a hepatic human cell line and CRL2522; human foreskin normal were also done. Results indicated that the peel extract was a better DPPH scavenger compared to NO. The extract was found to be rich in phenolic compounds with 416 mg of gallic acid equivalents per g dry weight of extract. However, the extract that rich in phenolic compounds extract was not found attributing towards the antiproliferative assay.

Keywords: fruit peel extract, antiproliferative, nitric oxide, free radical scavenging.

Introduction

Synthesis of phytochemicals in plants is resulted from a number of pathways in plant metabolism. Such pathways are important in maintaining the functions and confer protections to the plants (Larbat et al. 2014; Dong et al. 2016). Many medicinal plants are known to possess phytochemicals that are biologically active (Shayganni et al. 2016). Phenolic compounds, terpenoids, vitamins, and alkaloids are phytochemicals which have long history associated with health benefits and continues today as medicines. Thus, they are also called as bioactive compounds. The accumulation of bioactive compounds can be found in organs of the plant such as fruit, seed, flower, leaves, bark and root, but different in concentration. As an example, comparative phenolic compound profiles showed that total phenol content in ginseng fruit and leaves was higher than in ginseng roots (Chung et al. 2016).

Fruit peels have been found rich in bioactive compounds (Kabir et al. 2015), nevertheless this waste of restaurant, market, and byproducts of food and beverages industries are being thrown away or being fed to animals. However, noteworthy amount of bioactive component of therapeutic worth was recognized, thus encourages the study to find efficient ways to extract and evaluate the phytochemicals from the fruit peels to use its pharmacological properties (Salfarina 2016). For instance, the pear peel's anti-diabetic prospect of treatment for type II diabetes was proposed (Wang et al. 2015), whereas the development of encapsulated citrus by-product as nutraceutical with metalloproteinases inhibitory activity was reported (Lauro et al., 2015). Other studies reported antioxidant properties of phenolic compounds from fruit peel extracts (Contreras-Calderón et al. 2011; Fu et al, 2014; Sai-Ut et al. 2015). Frequently, the *in vitro* antioxidant assays are employed to evaluate the antioxidant activity of plant extract by evaluating the ability of extract to scavenge free radicals and/or molecules involved in the onset of oxidation process. Antioxidant rich extract was also reported exhibiting antiproliferative activity towards cancer lines (Moongkarndi et al. 2004; Wang et al. 2008).

This study was an initiative to adopt the interest of investigating the biological activity of fruit peel extract. The peels of mango (*Mangifera indica*, honeydew (*Cucumis melo*) and papaya (*Carica papaya*) obtained were the waste from the cafeteria of Universiti Teknologi MARA, Puncak Alam. Based on the reported literatures of fruit peel extract antioxidant activity, the preparation of fruit peel extract that consist of the combination of three different fruit peels was expected to provide a better source of natural antioxidant. Moreover, combinations of herbs are commonly observed among the practitioners of traditional medicine in countries of South East Asia. The objective of this research was to prepare a fruit peel extract by combining three fruit peels and to explore its free radical scavenging and anti-proliferative activities. Its total phenolic content was also measured by Folin–Ciocalteu assay.

Methodology :

Chemicals

Folin–Ciocalteu's phenol reagent, gallic acid, dimethyl sulfoxide (DMSO), 2,2-diphenyl-1-picryl-hydrazyl (DPPH), 95% ethanol analytical grade, CRL2522 cell, WRL68, HepG2 cell, human lung adenocarcinoma epithelial cells (A549), MEM medium, RPMI medium, fetal bovine serum (FBS), streptomycin, 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide(MTT), DMSO, penicillin, phosphate buffer solution (PBS), NaH2PO-K2HPO4 buffer, tyrosinase, L-tyrosine, L-DOPA solution, sodium nitroperruside, Griess reagent (sulphanilamide, naphtylethylenediamine dihydrochloride), ferrozine, ethanol, $FeCl_2$, EDTA.

Raw materials

The peels of mango, papaya and honeydew were collected from the Cafeteria of Universiti Teknologi MARA, Puncak Alam. The fruit peels were washed with tap water and oven-dried at 50 °C for 14 days. The dried fruit peels were weighed before being pulverized and stored at room temperature.

Preparation of fruit peel extract

The dried peels of papaya (29.16g), honeydew (97.51g) and mango (348.95g) were combined and macerated with 95% ethanol at 50°C. The solvent in the extract was removed with a rotary evaporator and water bath. The extract yield obtained was weighed and stored at 4°C until it was analyzed.

Determination of free radical scavenging activity

1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity

Free radical scavenging activity was evaluated by ability to scavenge DPPH; a stable synthetic free radical. The assay was adopted from (Salfarina et al. 2011) with a modification. Aliquot of 100 µl of 1,1-diphenyl-2-picrylhydrazyl (DPPH) in methanol (200µM) was pipette into 96 well microplate, followed by 100µl of extract prepared in ethanol. The reaction was allowed to incubate in 30 minutes at room temperature. Next, the absorbance was measured at 517 nm using microplate reader. The control was reaction mixture with ethanol substituting the extract. The DPPH radical scavenging activity was calculated by the following equation: Radical scavenging activity (%)= (Absorbance control –Absorbance sample)/ Absorbance control x 100. The scavenging activity of extract was expressed as the concentration necessary to scavenge free radical by 50% (IC_{50}). Gallic acid and quercetin with same concentrations were employed as positive controls.

Nitric oxide (NO) scavenging activity

Into a 96 microwell plate, 50µl of extract or control (ultrapure water) will be added into 50 µl of aqueous sodium nitroprusside (5 mM). The mixture will be incubated for 2 hours. Next 100 µl of Griess reagent (0.5% sulphanilamide in ultrapure water, 0.16% naphtylethylenediamine dihydrochloride in 20% acetic acid, 1:1) will be added and will be immediately read at 570nm. The nitric oxide scavenging activity will be calculated as below:
Scavenging activity (%)=(Absorbance control –Absorbance sample)/ Absorbance control x 100. The concentration of extracts necessary to scavenge 50% of the produced nitric oxide (IC_{50}) will be obtained from a graph of scavenging activity (%) against extract concentrations (Salfarina et al. 2011). Gallic acid and quercetin with same concentrations were employed as positive controls.

Determination of total phenolic content

Quantification of total phenolic content of extract was determined using Folin-Ciocalteu's phenol reagent. Phenolic compounds in the extract will form a blue color complex with Folin Ciocalteu reagent after adjusted with alkali. Briefly, 80µl of extract in methanol was pipette into 96 well microplate, followed by 100µl of 15% Folin Ciocalteau. Distilled water was added to adjust the volume to 200µl. The mixture was left for 5 minutes before addition of 100 µl Na_2CO_3 aqueous (0.105 g/ml). The absorbance of extract was measured at 756nm after incubation at 30˚C for 60 minutes. All determinations were performed in triplicate. Different concentrations of gallic acid (0.03, 0.06, 0.12, 0.25, 0.5 and 1 mg/ml) were used to prepare a standard curve. The concentration of total phenolic compounds in all extract was expressed as mg of gallic acid equivalents per g dry weight of extract using a linear equation (Salfarina et al. 2011).

Cell culture

The human lung adenocarcinoma epithelial cells A549, human liver cancer cell line HepG2 and WRL68 were obtained from the Pharmacology and Toxicology Research Laboratory, Faculty of Pharmacy Universiti Teknologi MARA, Puncak Alam. The subculturing was routinely performed when cells reached 80–90% confluency. A549 cell line, HepG2 and WRL68 was routinely sub-cultured in RPMI medium, MEM and DMEM medium, respectively. All cells were supplemented with 10% heat-inactivated fetal bovine serum (FBS), 200 µg/ml streptomycin and 500 µg/ml penicillin (Sigma, USA) at 37 °C in a humidified atmosphere and 5% CO_2 (Salfarina et al. 2015a).

Antiproliferative assay

The antiproliferative property of fruit peel extract against the A549, CRL 2522, HepG2 and WRL cells was evaluated by MTT assay. Briefly, 2×10^4 cells/cm^2 were seeded into 96-well plates and incubated overnight to ensure attachment of cells before fruit peel extract was added. The following day, cells were treated with 12.5, 25, 50, 100, 200 and 400µg/ml of extract and incubated for 24 h. Gallic acid or quercetin with same concentrations were employed as positive control, whereas DMSO was served as negative control. After indicated times, cells were washed with PBS and MTT solution (0.5 mg/ml, in PBS) was added to each well and incubated for another 4 h at 37 °C. Formazan crystals were dissolved in 100 µl DMSO, and the absorbance was determined at 570 nm using a spectrophotometer. The number of viable cells was then calculated as follows: Number of viable cells (%)= Absorbance of sample/absorbance of control x 100%. The antiproliferation activity of extract was expressed as the concentration necessary to inhibit the proliferation of cells by 50% (IC_{50}) (Salfarina et al. 2015a).

Statistical analysis

All the experiments were carried out in triplicate. The results were expressed as means and evaluated by analysis of variance (ANOVA) followed by Tukey's test. $P<0.05$ was regarded as statistically significant.

Results :

From the radical scavenging and antiproliferative assays, it was observed that the extract activities were in a concentration-dependent manner; thus a comparison of the extract, gallic acid or quercetin with all cells was determined from their IC_{50}. Table 1 summarizes the antioxidant and antiproliferative activity of fruit peel extract.

Table 1: IC_{50} of the free radical scavenging and anti-proliferative assays.

	IC_{50} (ug/ml)		
	Extract	Gallic acid	Quercetin
Free radical scavenging activity			
i) DPPH	38	2.27	5.81
ii) NO	94.8	79.4	94.5
Antiproliferative activity towards:			
i) HepG2	91.13	21.75	

ii)	WRL68	100.85	20.52	
iii)	A549	57.96		37.5
iv)	CRL2522	108.84		109.92

*Gallic acid was a positive control for HepG2 and WRL68 cells

*Quercetin was a positive control for A549 and CRL2522 cells

Discussion and Conclusion :

The antioxidant effect of the extract was assessed using the DPPH and NO scavenging assays. DPPH is a commercially available free radical that is widely used for a simple estimation of antioxidant activity. It has become a routine assay in determining an extract antioxidant activity. The decreased in absorbance after addition of extract results in a color change from purple to yellow, indicates the DPPH radicals are being scavenged by the extract through the donation of hydrogen to form the a stable DPPH molecule. Some pathological conditions showed that an increased in nitric oxide (NO) levels can result in inflammation and cancer. In this study, nitric oxide was generated from sodium nitroprusside. In aqueous solution at a physiological pH, sodium nitroprusside spontaneously generates nitric oxide which interacts with oxygen to produce nitric ions. The ions can be estimated using Griess reagent. In the assay, scavengers of nitric oxide reduce the production of nitric ions.

Results showed that the extract was preferentially scavenging DPPH than NO (Table 1). The scavenging activity of extract in both free radical scavenging assays was also found lower compared to gallic acid and quercetin. Gallic acid and quercetin are known phenolic compound with free radical scavenging activity. A lower value of IC_{50} exhibited by gallic acid and quercetin in DPPH assay indicated that gallic acid and quercetin was a better DPPH scavenger compared to NO. There was no significant difference in the NO scavenging activity of quercetin compared with the extract, both scavenged 50% of the nitric oxide produced by sodium nitroprusside at concentration of 94.5 and 94.8 µg/ml, respectively.

It was reported that mango peel extract exhibited stronger free radical scavenging ability on 1,1-diphenyl-2-picrylhydrazyl (DPPH) and the antiproliferative activity of mango peel extract was correlated with their phenolic and flavonoid content.(Kim et al. 2010) .In this study, the total phenolic compound of the extract was 416 mg of gallic acid equivalents per gram (GAE/g) dry weight of extract. This value was higher with respect to the previous report on extract of mango peel alone. For instance, two solvents were used to prepare a mango peel extract. Acetone extracted maximum amount of polyphenols followed by ethanol from both raw and ripe mango peels. Polyphenol contents in acetone extracts of raw and ripe varied from 55 to 110 mg GAE/g (Ajila et al. 2007). Thus, high total phenolic content obtained in this study was due to the combination of possible extracted phenolic compounds from the all peels used. In this study, the fruit peel extract was prepared by combining the dried honeydew, papaya and mango peels before being extracted with 95% ethanol at temperature 50°C. In addition, this extraction method was selected with an aim to extract more phenolic compounds from the peels (Paulucci et al. 2013).

Previously, the antioxidant and phenolic content of both seed and leaves of papaya were reported due to their application in traditional medicine, but not their peel. Concentration of phenolic compounds such as caffeic, cinnamic, chlorogenic, quinic, coumaric, vanillic, and protocatechuic acids, naringenin, hesperidin, rutin, and kaempferol were higher in young leaves compared to old leaves, whereas total phenolic content of young leaves was also higher than the seed extract (Gogna et al. 2015). Water extract was found effective to extract polyphenols from papaya leaves, however other than phenolic compounds, saponin was also found in papaya leaves, and its concentration was found higher than polyphenol in water, acetone, ethanol and methanol extracts of papaya leaves (Vuong et al. 2013). Therefore, the biological activity of papaya leaves not only could be contributed by polyphenols but also saponin. This study reported the antioxidant and total phenolic content of papaya and honey dew peels in combination with mango peel.

The antiproliferative effects of fruit peel extract on different cancer cell lines (A549,HepG2) along with normal cell line (WRL68, CRL2522) after 24 hours of incubation in the presence of extract, gallic acid or quercetin was evaluated by MTT assay. After the incubation period, the remaining living cells used their mitochondrial dehydrogenase enzyme to reduce MTT to an insoluble purple formazan product. The product can be measured using spectrophotometer to estimate relative cell proliferation. Gallic acid was a positive control for HepG2 and WRL68 cells, whereas quercetin was a positive control for A549 and CRL2522 cell. Both gallic acid and quercetin are phenolic compounds, classified as phenolic acid and flavonoids, respectively. Their antioxidant and antiproliferative properties are known. For instance, gallic acid induced apoptosis of rheumatoid arthritis fibroblast-like synoviocytes (RA FLS) by regulating the expressions of apoptosis-related proteins and inhibits pro-inflammatory gene expressions in RA FLS. It was suggested that gallic acid may offer a novel therapeutic or joint protection approach for the treatment of rheumatoid arthritis (Yoon et al. 2013). Quercetin which is the most common flavonois found in onion, apple and red wine induced morphological changes in MCF7 cells after 24 hour exposure and exhibiting significant antiproliferative compared to other flavonoids; myricetin and epicatechin (Rodgers and Grant 1998).

Among four cancer cell lines tested, the extract exhibited lowest IC_{50} values in A549 cells (53 µg/ml), followed by HepG2, WRL68 and CRL2522 cells. Frequently, few types of cell lines were employed to screen the cells that were susceptible to the extract. Moreover, different cells responded differently towards treatment, for instance No effect on the metabolic activity of MCF-7 cells after treatment with quercetin at 24 hour was found. But, 48 hours and 72 hours treatment of quercetin exhibited cytotoxic effect with IC_{50} values of 43.18µg/mL and 18.49µg/mL respectively (Salfarina et al. 2015b). A study established that the antiproliferative activity of an extract was attributed to the presence of phenolic compounds and found that the extract antiproliferative activity was higher compared to the activity of purified phenolic acid alone (Masci et al. 2016). This finding was in accordance with the observed ability of mango peel ethanolic extract in inhibiting proliferation of human cervix adenocarcinoma cells (HeLa), was attributed by the presence of quercetin 3-O-galactoside, mangiferin gallate, isomangiferin gallate, quercetin-3-O-arabinopyranoside, and mangiferin

in the extract (Kim et al. 2012). Nevertheless, it was found in this study that single compound as gallic acid and quercetin gave better IC_{50} values in antiproliferative assay than the extract, except for CRL2522 cell line. As an explanation of the superior activity of an extract compared to a single compound, frequently a synergistic effect of combinations of active compound is proposed. However, since quantification and identification of the extract phytochemical was not carried out in this study, it was unable corroborate the synergistic or antagonist effect that was responsible for the antiproliferative and antioxidant activities of the extract.

As a conclusion, a number of researches reported on the antioxidant, antiproliferative and total phenolic content of mango peel extract. However, this study reported for the first time the antioxidant, antiproliferative and total phenolic content of an extract prepared from the combination of dried papaya, honey dew and mango peels. As expected, the extract was found high in total phenolic content. The extract was most effective in inhibiting the proliferation of A549 cells. Nevertheless, the antioxidant and antiproliferative activity of the extract was found lower when compared with the activity of gallic acid and quercetin. The experimental results acquired in this study indicated that further work is needed to standardize and characterize the active principles in the extract. Understanding the inhibition of A549 cell proliferation by the extract will also be examined in the future, so that this unexploited natural resource can be a source of bioactive phenolics for nutraceutical or therapeutic ingredient. Moreover, the combination of dried peel in this study was made based on the availability of the peels. An appropriate proportion of dried peels should be made and the free radical and antiproliferative activities of that combination should be determined to get the best combination for the best activities.

Acknowledgements :
This study was funded by grants from the Malaysian Ministry of Higher Education, 600-RMI/RAGS 5/3 (101/2013) and 600-RMI/RAGS 5/3 (102/2013). We also would like to thank Research Management Centre, Universiti Teknologi MARA (RMC, UiTM). We have no potential conflict of interest including financial, personal or other relationships with other people or organizations.

References

1. Larbat R, Paris C, Le Bot J, Adamowicz S (2014) Phenolic characterization and variability in leaves, stems and roots of Micro-Tom and patio tomatoes, in response to nitrogen limitation. Plant Science 224:62-73
2. Dong X, Liu G, Wu X, Lu X, Yan L, Muhammad R, Shah A, Wu L, Jiang C (2016) Different metabolite profile and metabolic pathway with leaves and roots in response to boron deficiency at the initial stage of citrus rootstock growth. Plant Physiology and Biochemistry 108:121-131
3. Shayganni E, Bahmani M, Asgary S, Rafieian-Kopae M (2016) Inflammaging and cardiovascular disease: Management by medicinal plants. Phytomedicine 23:1119-1126
4. Chung I, Lim J, Ahn M, Jeong H, An T, Kim S (2016) Comparative phenolic

compound profiles and antioxidative activity of the fruit, leaves, and roots of Korean ginseng (Panax ginseng Meyer) according to cultivation years. Journal of Ginseng Research 40:68-75

5. Kabir F, Tow W, Hamauzu Y, Katayama S, Tanaka S, Nakamura S (2015) Antioxidant and cytoprotective activities of extracts prepared from fruit and vegetable wastes and by-products. Food Chemistry 167: 358-362
6. Salfarina R (2016) Importance of Drying Process in the Preparation of Plant Derived Crude Drug Extract. Research Updates in Medical Sciences (RUMeS) 1:1-2
7. Wang T, Li X, Zhou B, Li H, Zeng J, Gao W (2015) Anti-diabetic activity in type 2 diabetic mice and α-glucosidase inhibitory, antioxidant and anti-inflammatory potential of chemically profiled pear peel and pulp extracts (*Pyrus* spp.). Journal of Functional Foods 13:276-288
8. Lauro MR, Crasci L, Carbone C, Aquino RP, Panico AM, Puglisi G (2015) Encapsulation of a citrus by-product extract: Development, characterization and stability studies of a nutraceutical with antioxidant and metalloproteinases inhibitory activity. LWT - Food Science and Technology 62:169-176
9. Contreras-Calderón J, Calderón-Jaimes L, Guerra-Hernández E, García-Villanova B (2011). Antioxidant capacity, phenolic content and vitamin C in pulp, peel and seed from 24 exotic fruits from Colombia. Food Research International 44:2047-2053
10. Fu R, Zhang Y, Guo Y, Liu F, Chen F (2014) Determination of phenolic contents and antioxidant activities of extracts of *Jatropha curcas* L. seed shell, a by-product, a new source of natural antioxidant. Industrial Crops and Products 58: 265-270
11. Sai-Ut S, Benjakul S, Kraithong S,Rawdkuen S (2015) Optimization of antioxidants and tyrosinase inhibitory activity in mango peels using response surface methodology. LWT - Food Science and Technology 64: 742-749
12. Moongkarndi P, Kosem N, Kaslungka S, Luanratana O, Pongpan N, Neungton N (2004) Antiproliferation, antioxidation and induction of apoptosis by *Garcinia mangostana* (mangosteen) on SKBR3 human breast cancer cell line. Journal of Ethnopharmacology 90: 161-166
13. Shiow Y. Wang SY, Linda Bowman L, Min Ding M (2008) Methyl jasmonate enhances antioxidant activity and flavonoid content in blackberries (*Rubus* sp.) and promotes antiproliferation of human cancer cells. Food Chemistry 107:1261-1269
14. Salfarina R; Ken-ichi H; Nijsiri R (2011) Antioxidant, Antimicrobial and Cytotoxicity Activities of *Acacia farnesiana* (L.) Willd. Leaves Ethanolic Extract. Pharmacognosy Journal 2011:50-58
15. Salfarina R, Muhammad-Ashraf MS, Mohd-Radzi MNF, Eshak Z, Halim H (2015a) Efficacy and safety of *Hibiscus rosa sinensis* leaves from different solvent extracts 1st International Conference on Novel Frontiers in Pharmaceutical & Health Sciences 10-11 October 2015. Minal Campus, Ayodhya Bypass, Bhopal MP, India. Oral presentation
16. Hana Kim H, Jeong Yong Moon JY, Hyeonji Kim H, Dong-Sun Lee D, Moonjae Cho M, Hyung-Kyoon Choi H, Young Suk Kim YS, Ashik Mosaddik A, Somi Kim Cho SK (2010) Antioxidant and antiproliferative activities of mango (*Mangifera indica* L.) flesh and peel. Food Chemistry 121:429–436

17. Ajila CM, Bha SG,Prasada Rao UJS (2007) Valuable components of raw and ripe peels from two Indian mango varieties. Food Chemistry 102:1006–1011
18. Paulucci V, Couto R, Teixeira C, Freitas L (2013) Optimization of the extraction of curcumin from *Curcuma longa* rhizomes. Brazillian Journal of Pharmacognosy 23:94-100.
19. Gogna N, Hamid N, Dora K (2015) Metabolomic profiling of the phytomedicinal constituents of *Carica papaya* L. leaves and seeds by 1H NMR spectroscopy and multivariate statistical analysis. Journal of Pharmaceutical and Biomedical Analysis 115:74–85
20. Vuong QV, Hirun S, Roach PD , Bowyer MC, Phillips PA,Scarlett CJ (2013) Effect of extraction conditions on total phenolic compounds and antioxidant activities of Carica papaya leaf aqueous extracts. Journal of Herbal Medicine 3(3):104-111
21. Yoon C, Chung S, Lee S, Park Y, Lee S, Park M (2013) Gallic acid, a natural polyphenolic acid, induces apoptosis and inhibits proinflammatory gene expressions in rheumatoid arthritis fibroblast-like synoviocytes. Joint Bone Spine 80: 274–279
22. Rodgers EH and Grant MH (1998) The effect of flavonoids, quercetin, myricetin and epicathechin on the growth and enzyme activities of MCF7 human breast cancer cells. Chemico-Biological Interaction 116:213-228.
23. Salfarina R, Muhammad-Ashraf MS, Eshak Z (2015b). Imaging the in vitro cytotoxic effect of quercetin on MCF-7 cells. The 2nd International Conference on Advanced Pharmaceutical Research Faculty of Pharmacy, Rangsit University, Thailand. March 12, 2015. Oral Presentation.
24. Masci A, Coccia A, Lendaro E, Mosca L, Paolicelli P, Cesa S (2016) Evaluation of different extraction methods from pomegranate whole fruit or peels and the antioxidant and antiproliferative activity of the polyphenolic fraction. Food chemistry 202:59-69
25. Kim H, Kia H, Mosaddik A, Gyawali R, Ahn KS, Cho SK (2012) Induction of apoptosis by ethanolic extract of mango peel and comparative analysis of the chemical constitutes of mango peel and flesh. Food Chemistry 133(2): 416–422