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Cytotoxic activity and apoptosis induction of avocado *Persea americana* Mill. seed extract on MCF-7 cancer cell line

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ABSTRACT Avocado *Persea Americana* Mill. is a commercially important crop and studies have shown that the pulp may have benefits to cardiovascular health, dermatological health and possibly anti-cancer activity. Avocado seeds have several medicinal properties such as anti-hyperglycemic, antimicrobial, antioxidant and anti-inflammation. This study aim to evaluate the effect of avocado seed extract on viability and apoptosis of breast cancer cell line MCF-7. The anticancer effect was evaluated by cytotoxic test using MTT assay and the effect on apoptosis and cell cycle was examined by flow cytometry method. The cytotoxic test showed that chloroform extract had strong cytotoxic activity against MCF-7 cell lines with IC_{50} value of 94.87 µg/mL. Furthermore, the chloroform extract was partitioned with methanol and yield of soluble methanol fraction (FLM) and non soluble methanol fraction (FTLM). The cytotoxic activity of the methanol soluble fraction (FLM) and non soluble methanol fraction (FTLM) against MCF-7 cell lines was increased with IC_{50} of 34.52 and 66.03 µg/mL, respectively. Flow cytometry analysis using annexin-V and propidium iodide staining revealed that methanol soluble fraction could induce apoptosis and modulating the cell cycle arrest in MCF-7 cell. This research indicated that avocado seed has a potency to induce apoptosis and as anti-proliferative to MCF-7 cells lines.

KEYWORDS apoptosis; cytotoxic; MCF-7; *Persea americana*

1. Introduction

Cancer is one of the non-communicable disease which had high incidence and mortality rate worldwide. Ineffective treatments a long with high and expensive medications for cancer theraphy were lead to extensive research on drug discovery and drug development. It was generally accepted that fruit and vegetable consumption will be able to reduce the risk of human cancer (La Vecchia et al. 2001). The protective effect of fruit and vegetables is related to an amount of phytochemicals components. Avocado is one of commercial fruit that produce and consume worldwide. Central and South American countries dominate global avocado production. Mexico is the world's leading avocado producer while Indonesia noted as the second highest producer (USAID 2014).

Avocado *Persea americana* Mill. belong to Lauraceae family is an evergreen tree which native to Central America and presently have been cultivated worldwide. Avocado is grow in a wide range of climate from low land to mountainous region. Avocado is cultivated as delicious and nutritious fruits rather than as medicine. The fruit of avocado contains high percentage of lipids that reach up to 20% of dry weight (Takenaga et al. 2008). A part of nutritional and economical value of its fruits, several folk medicinal properties has been reported from the different organs of *P. americana*. Avocado is used in treating tumor in ethnomedicine and exhibits a chemoprotective effect on human cells (Paul et al. 2011).

One-half an avocado is a nutrient and phytochemical dense food consisting of dietary fiber, vitamins, minerals, and unsaturated fatty acid that have many health benefit (Dreher and Davenport 2013). Avocado seed is underutilized and represents a large portion of the fruit (Duarte et al. 2016). The seed amount more or less 16% of total avocado weight, and it is still an under-utilized resource (Ramos-Jerz et al. 2013). Phytochemical studies on avocado seeds have identified various classes of natural compounds such as phytosterols, triterpenes, fatty acids, furanoic acids, abscisic acid, proanthocyanidins, and polyphenols (Ding et al. 2007; Leite et al. 2009). Wang et al. (2010) have reported the presence of catechin, epicatechin, and A- and B-type procyanidin dimers and trimers, tetramers, pentamers, and hexamers in the seed of avocado.

Ethnopharmacological studies of the Aztec and Maya cultures have reported the use of decoctions of avocado seeds for the treatment of mycotic and parasitic infections.

Seeds have also been reported for use against diabetes, inflammation, and gastrointestinal irregularity (Dabas et al. 2013). The powdered form of avocado seed has been used for skin eruptions and to cure dandruff (Morton and Dowling 1987). Avocado seeds have more antioxidant activity and polyphenol content than the pulp (Nagaraj et al. 2010). Recent studies have demonstrated the anti-cancer, anti-diabetic, anti-inflammatory, blood pressure reducing, anti-microbial, insecticidal and dermatological activities of seed preparations. The anti-carcinogenic effects of avocado pulp have been investigated. Inhibition of PC3 and LNCaP prostate cancer cell lines was observed after treatment with an acetone extract of the pulp of Hass variety (Ding et al. 2009). In this study, it will observe the effect of soluble (MF) and non-soluble (NMF) methanolic fraction of avocado seed extract on the viability and apoptosis of breast cancer cell line MCF-7.

2. Materials and methods

2.1. Plant source

Fresh seeds of avocado were obtained from the Collection Garden of Medicinal Plant and Traditional Medicine Research and Development Centre, at the altitude of 1.200 m above sea level, with andosol type of soil. The fruits were harvested on July 2015, and after ripening in air temperature for six days than it was peel off to collect the seeds. The seeds were sliced into small and thin size, then drying in oven with 50°C of temperature for 3 x 24 h.

2.2. Extraction and fractionation

As 1000 g of dried powdered of seed was pulverized, then macerated in chloroform for three days and then filtered. The solvent was removed using a rotary evaporator at 50°C for 6–8 h. In our previous paper, the chloroform extract (EKBP) showed highest activity on MTT assay, therefore CE was chosen for the study (Widiyastuti et al. 2014). The chloroform extract was further partition with methanol and yielded soluble (FLM) and insoluble methanolic fraction (FTLM). All the extracts were examined for the chemicals profiles by spot test and TLC methods (Harborne 1987). FLM and FTLM were evaluated for its cytotoxic activity using MTT assay. The fraction which has more cytotoxic activity was used in this study to evaluate the capability of the extract to induce cell cycle arrest and apoptosis in MCF-7 cancer cell lines.

2.3. MTT assay of soluble (FLM) and insoluble methanolic (FTLM) fraction

The cytotoxic tests were carried out using the MTT assay method. MCF-7 were cultured maintained in Modified Eagle's medium (MEM) supplemented with 10% fetal bovine serum (FBS) and antibiotics (100 µg/mL penicillin and 100 µg/mL streptomycin) and cultured in CO_2 incubator containing 5% CO_2 at 37°C. The cells were seeded at a density of 8 x 10^5 cells in 96-well plates with MEM medium and incubated for 24 h. The cells were treated with extracts of various concentration such as 5, 20, 30, 60, 120, and 200 µg/mL in 0.1% DMSO for 48 h exposure time. After 48 h, 100 µL of a 1 mg/mL of solution of MTT in MEM was added to each well. The culture plates were incubated for 4 h at CO_2 incubator containing 5% CO_2 at 37°C. MTT was removed carefully and then stop solution (HCL in isopropanol) was added to each well and the plate was vigorously shaken to ensure that the blue formazan was completely dissolved. The absorbance was measured at 595 nm in automated plate reader (Elisa Reader Biorad) and percentage of growth inhibition was calculated using Equation 1.

$$\text{Inhibitory rate } \% = \frac{(\text{Absorbantion control} - \text{Absorbantion test})}{\text{Absorbantion control}} \times 100\% \quad (1)$$

IC_{50} was defined as the concentration of the plant extracts killing 50% of the cells. IC_{50} was determined for MCF-7 cell lines of both extracts.

2.4. Cell cycle evaluation

A number of 5 x 10^5 cells MCF-7 in 1 mL of culture medium EMEM grown in a six-well plate, and adapted for 24–48 h in a 5% CO_2 incubator temperature of 37°C. Once the cells was confluent, than it were treated by ½IC_{50} test sample concentration and IC_{50} (30 µg/mL). One of the well filled with the culture medium as a control. Observation and documentation of the cells under inverted microscope is done after 24–48 h of incubation. Each treatment was prepared a 15 mL conical. Cell culture media was transferred into conical, washed with 1x PBS and then transferred to the same conical. Cells were harvested with 150 mL of 0.25% trypsin-EDTA which incubated for 3 min. Cells were resuspensioned with 1 mL of culture medium. Remaining cells were taken with PBS and then transferred to a conical tube and centrifuged at 500 rpm for 4 min. The supernatant was discarded; the cell pellet was washed with 500 mL of cold PBS. Further, conical tube was centrifuged at 500 rpm for 4 min. The supernatant was discarded; the cells were fixed with a drop of 500 µL of 70% ethanol into conical tube than shaken slowly and stood for 30 min at room temperature. Conical tube was centrifuged around 2000 rpm for 2 min, than the alcohol removed and cells were washed with 500 µL of PBS and then centrifuged around 2000 rpm for 2 min. PI reagent (20 µL propidium iodide (Sigma) 1 mg/µL + 0.5 µL triton-x (Sigma) 100 + 1 µL RNase (Roche) 10 mg/µL in 500 µL of PBS for each sample) was added to the cell pellet, than resuspensioned and stored in a dark room for 20 min. The cell suspension was transferred into the new tube/micro tube then read and analyzed using BD flow cytometer Accuri C6.

2.5. Flow cytometry analysis of apoptosis

Cellular DNA content was determined by flow cytometry analysis of PI-labeled cells. A number of MCF-7 cells of 5 x 10^5 was seeded in 1 mL of culture medium EMEM

TABLE 1 Effect of FLM, FTLM and EKBP on MCF-7 cells viability.

Concentration (µg/mL)	Viability (%)		
	EKBP	FLM	FTLM
5	91.17 ± 6.20	90.74 ± 3.39	89.73 ± 9.26
20	83.50 ± 4.30	82.50 ± 5.98	78.23 ± 6.62
30	75.37 ± 1.40	57.29 ± 2.18	74.04 ± 3.73
60	61.35 ± 4.10	50.15 ± 3.41	62.05 ± 2.58
120	51.49 ± 2.30	7.80 ± 1.35	42.73 ± 4.37
200	5.76 ± 1.90	0.50 ± 1.41	13.66 ± 2.71
IC_{50}	94.87	34.52	66.03

grown in a 6-well plate, and adapted for 24–48 h in a 5% CO_2 incubator with temperature of 37°C. Once confluent, the cells were treated with ½IC_{50} and IC_{50} of concentration of avocado seed extract. One of the well was only filled by culture medium as a control. Observation and documentation of the cells under inverted microscope were done after 24–48 h of incubation. Each treatment was prepared a fruit conical 15 mL. Cell culture media is transferred in conical, washed with 1x PBS and then transferred to the same conical. Cells were harvested with 150 µL of 0.25% trypsin-EDTA and incubated for 3 min. Resuspension of cells with 1 µL culture medium. Remaining cells were taken with PBS and then transferred to conical and centrifuged 500 rpm for 4 min. The supernatant was discarded, the cell pellet was added with apoptosis reagent (FITC annexin 3 mL+3 mL of propidium iodide in 100 mL of buffer annexin), left in a dark room at room temperature for 10 min. Cells suspension was transferred to a tube then read and analyzed using BD flow cytometer Accuri C6.

2.6. Data analysis

Data analysis to calculate the IC_{50} value from single cytotoxicity assay, we plotted linear regression of concentration and percentage of cells viability using Excel MS Office 2013. Combination treatment was evaluated by calculating the Combination Index (CI) value with Compusyn software. The data obtained from flow cytometer was analyzed using BD Accuri C6 software.

3. Results

3.1. Chemical profiles of soluble and non-soluble methanolic fraction of avocado seed extract

Chemical analysis was conducted to determine the different compound contains in the extracts. Qualitative determination of chemical compounds of FLM, FTLM and EKBP extracts showed the different profiles of chromatogram Figure 1.

In the soluble methanolic fraction appeared five spots having Rf value between 0.4 to 0.9, and in the insoluble methanolic fraction appearing three spots on visualization with UV both 254 and 366 nm as well as with reagents.

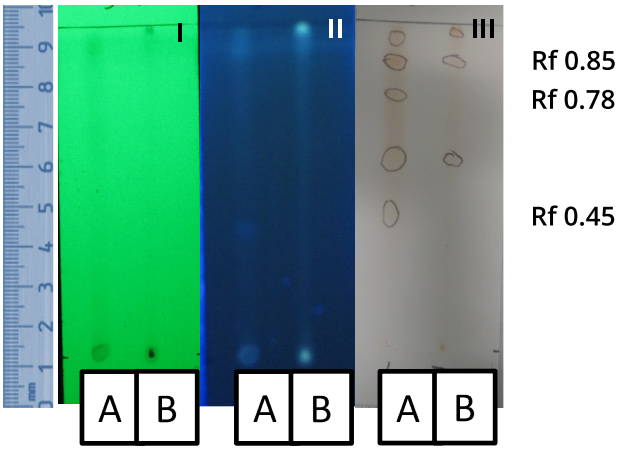

FIGURE 1 Chromatography profile of avocado seed extract. A: soluble methanolic fraction (FLM); B: non soluble methanolic fraction (FTLM) extract, developed in hexane:ethyl acetate (5:5), visualization by UV 254 nm (I), UV 366 nm (II) and sulphuric acid 5% in methanol and heated at 100°C for 15 min (III).

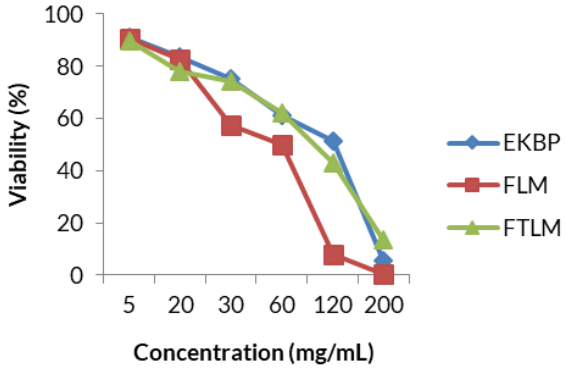

FIGURE 2 Mortality of MCF-7 cells after treatment with avocado seed extract.

In this study, the thin-layer chromatographic profile (Figure 1) of the non-soluble fraction indicates the presence of non-polar compound with the Rf value more than 0.5. The insoluble methanolic fraction of avocado seeds showed only less spot on TLC which indicates that almost all of the compounds have been dissolved in methanol.

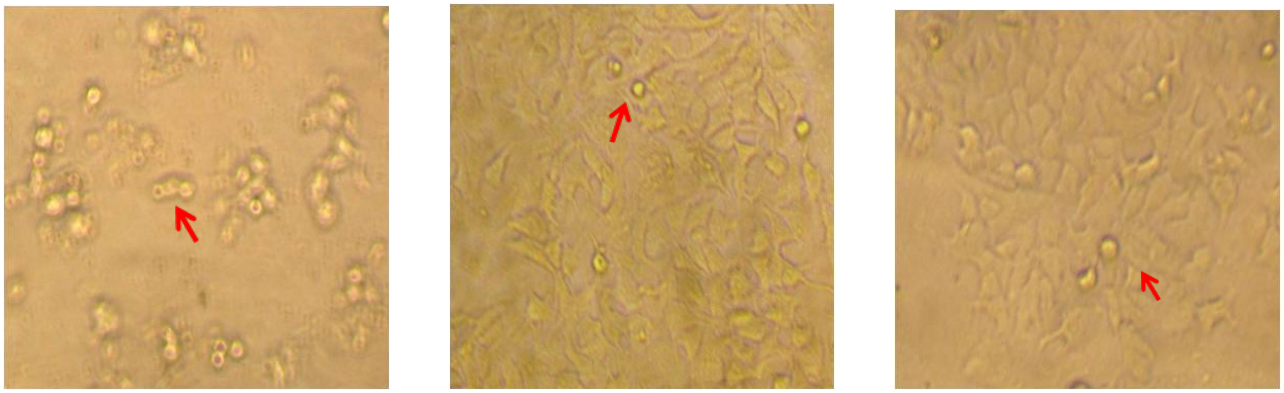

FIGURE 3 Morphological changes of MCF-7 cells treated by FLM (a), FTLM (b), and EKBP (c) at the concentration of 30 µg/mL (the red arrows indicated the cell death). The picture was taken under inverted microscope with 200× magnification.

3.2. *Inhibitory effect of methanolic fraction of avocado seed extraction MCF-7 cells growth*

The chloroform extract was partition with methanol and yielded soluble (FLM) and insoluble methanolic fraction (FTLM). The methanolic soluble fraction (FLM) and FTLM were evaluated for its cytotoxic activity using MTT assay. Cytotoxic test showed IC_{50} of FLM and FTL Mon MCF-7 cells growth resulted the decreasing value of IC_{50} compare to chloroform extract of avocado seed (EKBP) (Table 1).

Profiles obtained from cell viability by MTT test showed that the FLM and FTLM of avocado seed in general may decrease the viability of MCF-7 cells compared to chloroform extract. The effect of all avocado seed extracts were showed by decreasing of cell viability with dose dependent manner. Cells treated with FLM, FTLM and EKBP for 48 h resulted in growth inhibition and cell death in a dose dependent manner (Figure 2).

Cytotoxic effect is the effect depends on the concentration level of materials where the greater concentration of materials so the greater of the cytotoxic effect levels (Wahyuningsih et al. 2015). From the previous evaluation, avocado seed chloroform extract (EKBP) shown the highest inhibitory activity (Widiyastuti et al. 2014). Active compounds contained in chloroform extract of avocado seed are estimated to have the potential cytotoxic activity of its value IC_{50} lower than 100 µg/mL (Table 1).

Furthermore, the effect of FLM and FTLM on cell viability also observed by the morphological changes of MCF-7 cell line. FML causes more cell deaths than FTLM which characterized by morphological changes in the form of cell shrinkage and cell extension. At a concentration of 30 µg/mL, FLM could kill more than 50% of MCF-7 cells (Figure 3).

3.3. *Effect FLM and FTLM on cell cycle of MCF-7 cancer cell lines*

In this study, MCF-7 breast cancer cell lines was used to evaluate the capability of avocado seed methanolic fraction of chloroform extract to induce apoptosis and effect on cell cycle. Observation of cell cycle profile was performed on the soluble and insoluble methanolic avocados seed extract (Figure 3). Treatment of FLM and FTLM extracts did not affect the overall cell cycle profile, but induced an increase in the number of cells in subG1 phase (Figure 4).

Percentage of cell counts in each cycle phase can be seen in Table 2. Both extract are not affected the cell cycle of MCF-7 significantly. The contrast was the FLM extract able to increase the number of cells in SubG1/G0 phase from 5.1% to 21.9% after 48 h of incubation which reflects the occurrence of apoptotic event.

3.4. *FLM and FTLM of avocado seed induced apoptosis in MCF-7 cell lines*

To understand cell death mechanism in subG1 population, whether it was mediated through apoptosis, it was stained treated cells with propidium iodide–annexin and subjected to flow cytometry analysis. The results showed the apoptotic population was significantly increased in MCF-7 cells treated by both avocado seed extract of FLM and FTLM as well (Figure 5).

4. Discussion

Avocado are fruits that are usually consumed in our diet and also have compounds that are essential for health, including terpenoid and phenolic substances. Avocado seed is discarded and considered as a waste of product. From the previous study catechin and epicatechin were isolated from a methanolic extract of avocado seed showed antioxidant activity in an AMVN-induced methyl linoleate peroxidation assay (Matsusaka et al. 2003). Avocado seed also rich in polyphenols with antioxidant and antimicrobial power (Rodríguez-Carpena et al. 2011). The chloroform and methanolic soluble fraction of avocado seed have a different compounds indicated by a number and Rf value of the spots.

Research showed that qualitative determination of chemical group compound of avocado seed chloroform extract contained alkaloid, saponin, and terpenoid, while

the methanolic extract contained alkaloid, saponin, tanin, terpenoid, and flavonoid (Widiyastuti et al. 2014). The differences polarity of the various solvents are perhaps responsible for the differences in the solubility of plants active compound, therefore affect in degree of activity. Alkaloid, flavonoid and terpenoid are the chemical compound groups which possess cytotoxic activity, inhibited cell growth and induced apoptosis in various lines of cancer (Taraphdar et al. 2001). The solvent of extraction has been shown to be an important factor in the extraction of bioactive compounds of avocado seed that lead to different activities. Isolated compound of avocado seed ethanolic extract that identified as triterpenoid was able to inhibit the viability of MCF-7 cancer cell line with IC_{50} value of 62.43 µg/mL (Abubakar et al. 2017).

The partition of avocado seed chloroform extract with methanol yielded soluble (Figure 1). The methanolic soluble fraction has contained more polar compound compare to chloroform extract, indicate by the lower of Rf value of the spots. That means the methanolic soluble fraction has more cytotoxic activity since the existence of the polar compounds. According to Elmore et al. (2002), the highest concentration of a test agent in cytotoxicity evaluation should be less than 1.000 µg/mL or 1.000 µM. If none of the concentrations of test agents exhibited cytotoxic effect in excess of 50% of cell populations, the test agent is considered non-toxic against the tested cell line. In the present study, FLM and FTLM extracts at concentrations of 100 µg/mL could reduce the viability of MCF-7 cells to less than 50% (Figure 3). Thus, both extracts were considered toxic against MCF-7 cells. From the results obtained it is known that the compounds responsible for the cytotoxic activity of avocado seeds are more polar.

The measurement of the viability of cancer cell line is one of the parameter to evaluate the anticancer potency of avocado seed extract. Moreover the effect of the extract on cell growth and the induction of apoptosis are also important parameters to evaluate the respective anticancer properties of avocado seed extracts. Therefore, cell cycle analysis was conducted to determine the influence of avocado seed extract on the cell cycle behavior and apoptosis initiation by flow cytometry method. Based on cell cycle profile with flow cytometry, treatment of extract induces apoptosis (sub phase G1 and G2/M) without modulating the cell cycle. FLM was induce sub-G1 phase arrest (21.9%) which reflecting of apoptotic cell death population as compared to FTLM and control cell which only 5.1% and 3.7% respectively (Table 2). Ding et al. (2009) suggest that the chemical compounds present in FLM extract can inhibit the growth of oral epithelial of cancer cell line with the target of protein regulation in the cell cycle. Apoptosis or cell death progam, is a cellular process that occurs in normal or pathological of physiological conditions. Disregulated form of molecular sig-

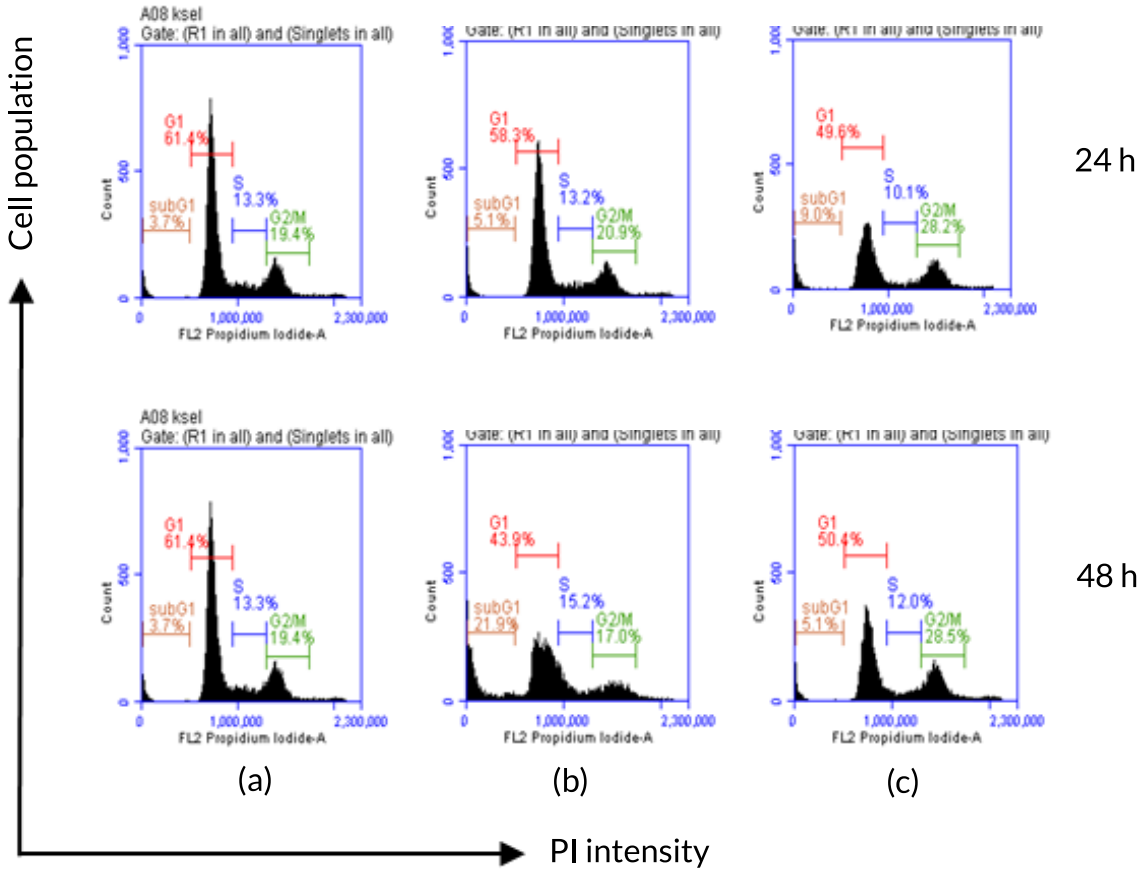

FIGURE 4 Cell cycle analysis of MCF-7 cells: the cell cycle distribution of MCF-7 cells after treatment with soluble methanolic extract (b) and insoluble methanolic fraction (c) compared with the control cells (a) of 30 µg/mL during 24 and 48 h of incubation.

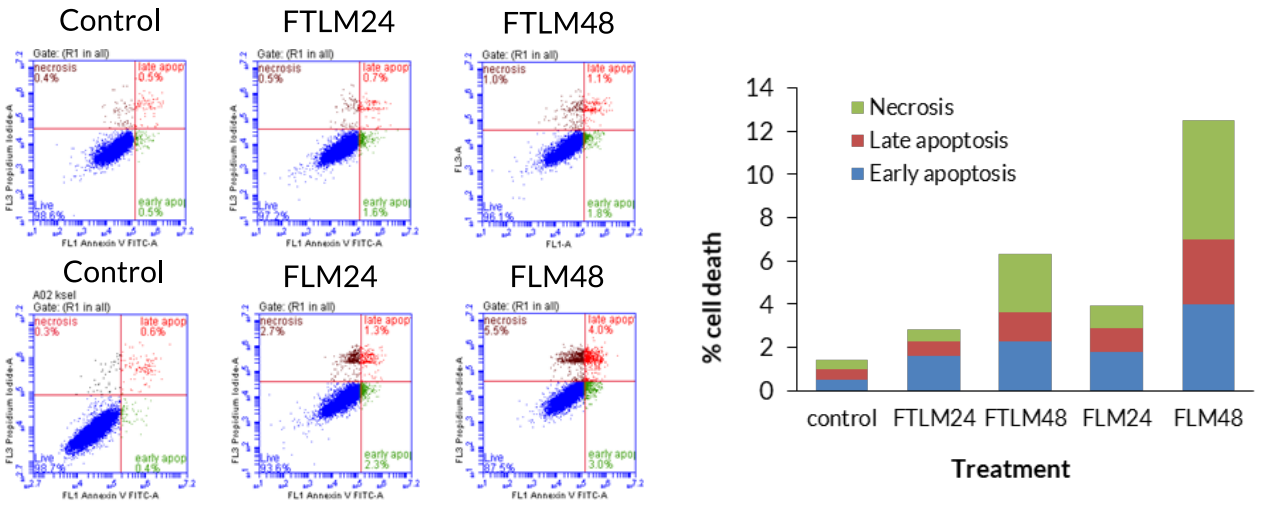

FIGURE 5 FLM and FTLM significantly induced apoptosis in MCF-7 cell. Cells were treated for 24 h and stained with annexin V-PI; each sample was subjected to flow cytometer in triplicate. The graphic showed statistical analysis results for apoptosis induction for each treatment.

TABLE 2 Percentage of cells in each phase due to the treatment of FLM and FTLM compared with cell control (Ks) on 24 and 48 h of observation time.

No.	Treatments	% distribution			
		SubG1	G1	S	G2/M
1	Control	3.7	61.4	13.3	19.4
2	FLM 24 h	5.1	43.9	15.2	17.0
3	FLM 48 h	2.9	58.3	13.2	20.9
4	FTLM 24 h	9.0	49.6	10.1	28.2
5	FTLM 48 h	5.1	50.4	12.0	28.5

naling of apoptosis resulting in a proliferation imbalance and cell death which plays an important role incarcinogenesis events. Therefore, induction of apoptosis is one of the mechanisms which contribute importantly in cancer therapy (Wong 2011). Therefore, the molecular mechanism of avocado seed extract in inducing apoptosis of MCF-7 cancer cells needs to be further studied.

Cell apoptosis was also increased by FLM dose-dependently (Figure 5). A previous study revealed that *Persea americana* Mill var. Hass (Lauraceae) was inhibited viability and induce apoptosis in prostate and oral epithelial cancer cell lines (D'Ambrosio et al. 2011; Ding et al. 2009).

5. Conclusions

The methanol soluble fraction of avocado seed extract has the highest cytotoxic activity on MCF-7 cell lines because it has the lowest IC_{50} value of 34.52 mg/mL.The methanol soluble fraction (FLM) and the insoluble methanolic fraction(FTLM) did not significantly affect the cell cycle but FLM extract was increase the accumulated of cell in the subG1 and G2/M phase that indicate the occurance of apoptotic event.

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Authors' contributions

YW, SR and SW design the study, YW, SR and SW carried out the laboratory work, RP, SR, and SW were validated the result of analyzed data, YW, RP, SR and SW wrote the manuscript. All the author read and approved the final version of the manuscript.

Competing interests

The authors wish to disclose that there are no under financial, general, and institutional competing interests and such as those defined above or others that may be perceived to influence the results and discussion reported in this paper.

References

Abubakar ANF, Achmadi SS, Suparto IH. 2017. Triterpenoid of avocado (*Persea americana*) seed and its cytotoxic activity toward breast MCF-7 and liver HepG2 cancer cells. Asian Pac J Trop Biomed 7(5):397–400. doi:10.1016/j.apjtb.2017.01.010.

Dabas D, Shegog RM, Ziegler GR, Lambert JD. 2013. Avocado (*Persea americana*) seed as a source of bioactive phytochemicals. Curr Pharm Des 19(34):6133–6140. doi:10.2174/1381612811319340007.

D'Ambrosio SM, Han C, Pan L, Kinghorn AD, Ding H. 2011. Aliphatic acetogenin constituents of avocado fruits inhibit human oral cancer cell proliferation by targeting the EGFR/RAS/RAF/MEK/ERK1/2 pathway. Biochem Biophys Res Commun 409(3):465–469. doi:10.1016/j.bbrc.2011.05.027.

Ding H, Chin YW, Kinghorn AD, D'Ambrosio SM. 2007. Chemopreventive characteristics of avocado fruit. Semin Cancer Biol 17(5):386–394. doi:10.1016/j.semcancer.2007.04.003.

Ding H, Han C, Guo D, Chin YW, Ding Y, Kinghorn AD, DÁmbrosio SM. 2009. Selective induction of apoptosis of human oral cancer cell lines by avocado extracts via a ROS-mediated mechanism. Nutr Cancer 61(3):348–356. doi:10.1080/01635580802567158.

Dreher ML, Davenport AJ. 2013. Hass avocado composition and potential health effects. Crit Rev Food Sci Nutr 53(7):738–750. doi:10.1080/10408398.2011.556759.

Duarte PF, Chaves MA, Borges CD, Mendonça CRB. 2016. Avocado: characteristics, health benefits and uses. Cienc. Rural 46(4):747–754. doi:10.1590/0103-8478cr20141516.

Elmore E, Siddiqui S, Desai N, Moyer MP, Steele VE, Redpath JL. 2002. The human epithelial cell cytotoxicity assay for determining tissue specific toxicity: method modifications. Methods Cell Sci 24(4):145–153. doi:10.1023/A:1024453300493.

Harborne J. 1987. Metode Fitokimia: Penuntun Cara Modern Menganalisis Tumbuhan [Methods in phytochemistry: A guide on modern plant analysis]. Bandung: ITB. Translated by Padmawinata, K & Soediro, I.

La Vecchia C, Altieri A, Tavani A. 2001. Vegetables, fruit, antioxidants and cancer: a review of Italian studies. Eur J Nutr 40(6):261–267. doi:10.1007/s394-001-8354-9.

Leite JJG, Brito EHS, Cordeiro RA, Brilhante RSN, Sidrim JJC, Bertini LM, Morais SMd, Rocha MFG. 2009. Chemical composition, toxicity and larvicidal and antifungal activities of *Persea americana* (avocado) seed extracts. Rev Soc Bras Med Trop 42(2):110–113. doi:10.1590/s0037-86822009000200003.

Matsusaka Y, Kawabata J, Kasai T. 2003. Antioxidative constituents in avocado *persea* americana Mill.) seeds. Nippon Shokuhin Kagaku Kogaku Kaishi 50:550–552. doi:10.3136/nskkk.50.550.

Morton J, Dowling C. 1987. Fruits of warm climates. Michigan University: JF Morton.

Nagaraj M, Sandhya V, Supriya G, Manju R, Kumari P, Bole S, Lalitha V, Kiran B. 2010. Antioxidant and antibacterial activity of avocado (*Persea gratissima* Gaertner.) seed extract. World Appl Sci J 9(6):695–698.

Paul R, Kulkarni P, Ganesh N. 2011. Avocado fruit (*Persea americana* Mill) exhibits chemo-protective potentiality against cyclophosphamide induced genotoxicity in human lymphocyte culture. J Exp Ther Oncol 9(3):221–230.

Ramos-Jerz MDR, Villanueva S, Jerz G, Winterhalter P, Deters AM. 2013. *Persea americana* Mill. seed: fractionation, characterization, and effects on human keratinocytes and fibroblasts. Evid Based Complement Alternat Med 2013:1–12. doi:10.1155/2013/391247. Article ID 391247.

Rodríguez-Carpena J, Morcuende D, Estévez M. 2011. Avocado by-products as inhibitors of color deterioration and lipid and protein oxidation in raw porcine patties subjected to chilled storage. Meat Sci 89(2):166–173. doi:10.1016/j.meatsci.2011.04.013.

Takenaga F, Matsuyama K, Abe S, Torii Y, Itoh S. 2008. Lipid and fatty acid composition of mesocarp and seed of avocado fruits harvested at northern range in Japan. J Oleo Sci 57(11):591–597. doi:10.5650/jos.57.591.

Taraphdar AK, Roy M, Bhattacharya RK. 2001. Natural products as inducers of apoptosis: Implication for cancer therapy and prevention. Curr Sci 80(11):11.

USAID. 2014. The U.S. market for Avocado. Technical Report Market Survei #19. USAID; [cited 2017 January 28]. [place unknown]. Available from: http://pdf.usaid.gov/pdf_docs/PA00KP28.pdf.

Wahyuningsih MSH, Wijayanti MA, Budiyanto A, Hanafi M. 2015. Isolation and identification of potential cytotoxic compound from kembang bulan (*Tithonia diversifolia* (Hemsley) A Gray) leaves. Int J Pharm Pharm Sci 7(6):298–301.

Wang W, Bostic TR, Gu L. 2010. Antioxidant capacities, procyanidins and pigments in avocados of different strains and cultivars. Food Chem 122(4):1193–1198. doi:10.1016/j.foodchem.2010.03.114.

Widiyastuti Y, Pratiwi R, Riyanto S, Wahyuono S. 2014. Chloroform and methanolic extract of avocado seed (*Persea americana* Mill.) against MCF-7 cancer cell line. In: W Jokopriyambodo, Y Widyastuti, S Wahyono, R Mujahid, D Subositi, editors. Proceedings of the International Symposium on Medical Plants and Traditional Medicine. volume XLVI. Lembaga Penerbit Badan Penelitian dan Pengembangan Kesehatan. p. 598–607.

Wong RSY. 2011. Apoptosis in cancer: from pathogenesis to treatment. J Exp Clin Cancer Res 30:87. doi:10.1186/1756-9966-30-87.