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Research Article

CYTOTOXIC ACTIVITY OF AVOCADO SEEDS EXTRACTS (*Persea americana* MILL.) ON T47D CELL LINES

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ABSTRACT
Avocado (*Persea americana* Mill.) is a widely grown plant that had been studied for several activities such as antioxidant and chemopreventive. The aims of this study were to analyze the secondary metabolite compounds contained in avocado seeds and to determine cytotoxic effect from aqueous and ethanolic extracts of avocado seeds against T47D breast cancer cell lines. IC_{50} value obtained by using MTT assay on aqueous extract, ethanolic extract, and doxorubicin hydrochloride were 560,2 µg/mL, 107,15 µg/mL and 0,26 µg/mL, respectively. Phytochemical screening test had detected the alkaloids, glycosides, phenols, and saponins as chemical groups in avocado seeds extracts.
Keywords: avocado seeds, cytotoxic effect, polar extracts, T47D cell line.

INTRODUCTION

Trend of increasing prevalence of breast cancer can not be avoided due to the death rate of cancer. Chemotherapy had caused many uncomfortable side effects for the patient, the costs are very expensive and the degree of sucess has not been satisfactory. It is therefore necessary to explore herbs as an alternative medicine to fight cancer. One of the plants that is potential to be developed as anticancer is avocado. One of the experiment[1] showed that avocado seeds were toxic to the method of Brine Shrimp Lethality Test/BST (LC_{50} below 1000 ug/ml). Based on the results of previous studies[2,3], LC_{50} values obtained from the avocado seeds extract was less than 1000 mg/L. This value indicated that the avocado seeds were toxic. Therefore, the avocado seed extract can be further investigated to determine the cytotoxic effect of ingredients that can be developed as an anticancer drug. Total antioxidant capacity of avocado had been investigated by using the method of FRAP (Ferric Reducing Ability of Plasma)[4], which indicated that the avocado seeds had a higher antioxidant activity than fruits and leaves. It was also proved that asetogenin from acetone extracts of avocado seeds was cytotoxic[5]. Avocado seeds are richer in phenolic compounds than the leaves and fruits[5] which are potential as an anticancer agent. Our study aimed at evaluating the cytotoxic effect of *Persea americana* Mill. seeds extracts against T47D cell lines.

MATERIAL AND METHODS

Materials

Ethanol (Brataco Chemika, Indonesia), distilled water (Brataco Chemika, Indonesia), MTT (3-[4, 5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) (Invitrogen, USA), dimethyl sulfoxide/DMSO (Biobasic, Canada), T47D cell lines (ATCC, USA), micro 96 well plates (Nunc, Denmark).

Plant collection

Parts of the plant that were used as the sample were brown avocado seeds. The plants were determined at Indonesian Institute of Sciences, Biological Research Center, Cibinong. Avocado seeds that had been collected from Bogor Indonesia, sorted, washed, dried, and used as a crude drug powder through 60 mesh. Crude drug powder with distilled water and then macerated for 24 hours, filtered, and then concentrated by rotary vacuum evaporator at a temperature of 60ºC to obtain a viscous extract, and then weighed and calculated on the weight of crude drug rendemennya early. Cells used in this study was called T47D cell line, developed and grown/proliferated on media in laboratory of LAPTIAB Agency for the Assessment and Application of Technology (BPPT), Serpong.

In vitro cytotoxicity assay

T47D cells were seeded into 96-well plates at a density of 5 x 10^4 cells/well and left to attach to the plates for 48 hours. Different concentrations of petroleum extract (100 µL) were added to the T47D cancer cells, seeded in 96-well microtiter and incubated at 37ºC for 24 hours. At the end of the treatment, 100 µl of MTT [(3,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)] was added to each well and the microtiter plate were incubated for 4 hours at 37ºC. Finally, SDS (100 µl) was added to each well, after each absorbance was read at 570 nm on ELISA microplate reader. Experiments were carried in duplicate[7].

Phytocemical Screening

Phytochemical screening with some chemical reagents for alkaloids, flavonoids, triterpenoids/steroids, glycosides, saponins and polyphenols had been done on aqueous and ethanolic extracts [8].

RESULTS

Data obtained from the results of the ELISA reader in the form of absorbance readings of each pitting converted into a living cell percentage (% proliferation). The principle of data processing in this study were between the percentage of live cells correlate with the concentration of the extract. The measurement results using the ELISA reader T47D cells showed that the percentage of survival continues to decline comparable to the increase in the concentration of a given

extract. IC_{50} values in the cytotoxicity assay of aqueous extract on T47D cells was 560,2 µg/mL meanwhile ethanolic extract was 107,15 µg/mL (Figure 1 and 2), whereas the cancer drug doxorubicin HCl at 0,26 µg/mL (Figure 3). The results of the data analysis by one-way ANOVA statistical test showed a difference between the treatment sample.

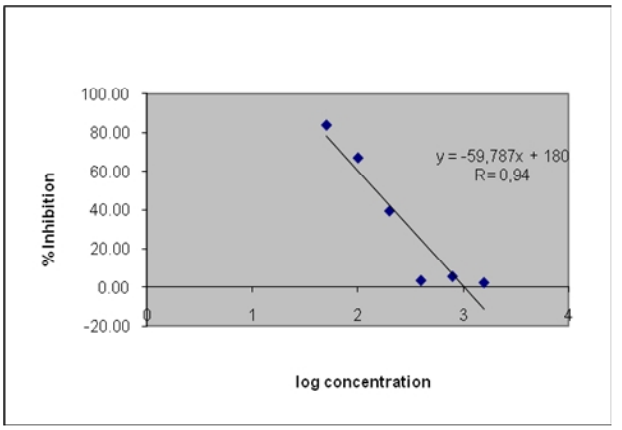

Figure 1: Relationship between log concentration of aqueous extract on inhibition percentage of T47D cells

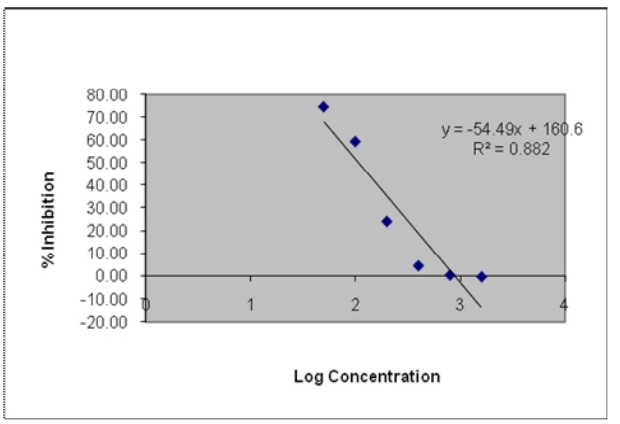

Figure 2: Relationship between log concentration of ethanol extract on inhibition percentage of T47D cells

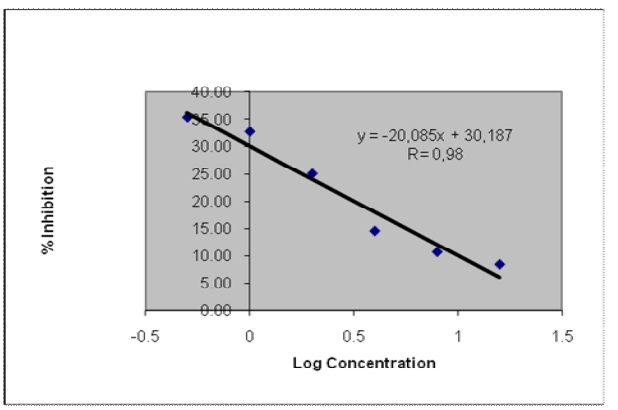

Figure 3: Relationship between log concentration of doxorubicin HCl on inhibition percentage of T47D cells

Table 1: Absorbance of Aqueous Extract of Avocado seeds

Concentration (µg/mL)	Absorbance
50	0,551 ± 0,048
100	0,462 ± 0,004
200	0,315 ± 0,073
400	0,123 ± 0,023
800	0,134 ± 0,002
1600	0,117 ± 0,003

Table 2: Absorbance of Ethanol Extract of Avocado seeds

Concentration (µg/mL)	Absorbance
50	0,503 ± 0,043
100	0,422 ± 0,051
200	0,234 ± 0,001
400	0,129 ± 0,001
800	0,107 ± 0,002
1600	0,103 ± 0,002

Table 3: Absorbance of Doxorubicin HCL

Concentration (µg/mL)	Absorbance
0,5	0,301 ± 0,011
1	0,286 ± 0,009
2	0,243 ± 0,008
4	0,183 ± 0,006
8	0,161 ± 0,012
16	0,148 ± 0,013

Table 4: Phytocemical Screening of Avocado seeds extracts

No.	Chemical Groups	Results	
		Aqueous extract	Ethanolic extract
1.	Alkaloid	+	+
2.	Flavonoid	+	+
3.	Glycoside	+	+
4.	Steroid/triterpenoid	-	-
5.	Saponin	+	+
6.	Polyphenol	+	+

DISCUSSION

Based on the reference[9], the extract with IC_{50} values ≤ 100 µg/mL has antiproliferative potency. It can be said that ethanolic extract was potential to inhibit the growth of cancer cell but the aqueous extract was not. This activity can be related due to the presence of polar groups of active compounds that are not optimally extracted in the water, which possible more soluble in organic solvents than aqueous solvent so that the assay showed better results in organic solvent extract such as ethanol than in aqueous extract. Based on the results of the phytochemical screening by TLC investigation, samples were identified the presence of compounds alkaloids, phenols, glycosides and saponins in the aqueous extract and ethanolic extract of *Persea americana* Mill seeds. It revealed the presence of polar groups in the extracts of avocado seeds which could be responsible for cytotoxic activity.

CONCLUSION

Based on the results of cytotoxicity assay of avocado seeds extracts against T47D breast cancer cells, aqueous extract obtained IC_{50} values of 560,2 µg/mL, the ethanolic extract 107,15 and IC_{50} values of doxorubicin HCl at 0,26 µg/mL. Based on the reference[9], aqueous extract of avocado seeds did not inhibit the growth of breast cancer cells T47D (> 100 µg/mL) but the ethanolic extract did. Based on the results of the chemical compounds test through TLC phytochemical screening, the results showed the presence of alkaloids,

phenols, glycosides and saponins in the aqueous extract and ethanolic extract of *Persea americana* Mill seeds.

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