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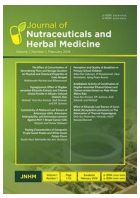

Cytotoxic Activities of Ethanol Extract, Nonpolar Semipolar, and Polar Fractions of *Dioscorea esculenta* L.) on MCF7 Cancer Cell

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Abstract

One of the diseases that have a high contribution to the death of women in the world is breast cancer. It leads to the research and exploration to find its cure. This study is conducted to know the anti-cancer activity on ethanol extract, nonpolar, semi polar and polar fractions of lesser yam (*Dioscorea esculent* L.) herbs. It also aims to find out the content of compound on semi polar fraction *Dioscorea esculent* L. herbs. The extraction was carried out using maceration technique with 96% ethanol as solvent. Then, the fractionation was done using vacuum liquid chromatography method with n-hexane and acetone as mobile phase with the ratio of 9:1, 8:2, 7:3, 6:4, and 5:5. After that, the phytochemical test was undertaken using the thin layer chromatography with n-hexane and acetone as mobile phase with proportion 7:3. Meanwhile, the cytotoxic test was conducted using MTT assay method with IC_{50} calculation was counted using linear regression between the log concentration and the percentage of living cells. The semi polar fraction of *Dioscorea esculent* L. herbs has IC_{50} value of 632,42 µg/mL, whereas the ethanol extract, nonpolar and polar fractions have the IC_{50} value > 1000 µg/Ml, Furthermore, the compound of the semi polar fraction of *Dioscorea esculent* L herbs are saponins, terpenoids, alkaloids, and phenolic.

Keywords: cytotoxic; d*ioscorea esculent*; lesser yam herbs; MCF7; MTT assay.

1. INTRODUCTION

Breast cancer is one cause of death in women because the cells can have metastasis (Meiyanto *et al.*, 2006). The cause of breast cancer is because breast's cells can avoid the mechanism of cell death so cell division can not be controlled (Meiyanto *et al.*, 2011). Breast cancer that one of its cells used as a trial model is MCF7 or Michigan Cancer Foundation-7. MCF7 cancer cells produce PgP and Bcl-2 proteins that initiate excessive anti-apoptosis, and do not produce caspase-3 so that apoptotic processes can be avoided (Hermawan and Meiyanto, 2010). Programmed cell death is important in biological processes; its activity can be affected by various plant extracts.

One of the plant extracts studied in its content and its usefulness as a drug is gembili (*Dioscorea esculent* L.) According to Mar'atirrosyidah (2015), gembili contains bioactive compounds such as dioscorin, phenol, and also dysgenic with levels of 2.77 mg / 100 grams of material. In the pharmaceutical industry, dysgenic is an important compound as a source of natural steroid hormones (Chiang *et al.*, 2007). Diosgenin is also reported to have anti-inflammatory activity, cytotoxic activity, anti-tumor, anti-fungal, immunoregulation, hypoglycemic and cardiovascular (Marie-Aleth, 2005; Wagner, 2000). In addition, several Dioscorea species have been used in traditional Chinese medicine as anticancer agents, gastropathic protectors, heart, as curative agents and anti-rheumatic agents (Sautour, 2007).

According to research conducted by Soetoko and Sumarno (2012), ethanol extract of gembili bulb has cytotoxic activity against T47D cancer cell with IC_{50} 39,61 µg/mL Methanol extract of gembili leaves has cytotoxic activity against Vero cell with IC_{50} 111,8 mg/L (Muzaimah *et al.*, 2010). Nevertheless, research on cytotoxic activity of gembili plants is still limited, so research is done to find out the cytotoxic activity of ethanol extract, nonpolar fraction, semi polar, polar gembili herb against MCF7 cancer cells, and to know the compound content in potentially anticancer samples.

2. METHODS

2.1. Extraction and Fractination

The leaves and stems of the gembili plants are cut, washed with clean running water, then dried using a drying cupboard. After dry, the herb gembili mashed with a blender. A total of 600 grams of herb gembili powder soaked with 4.5 L ethanol 96% overnight, then done remaserasi as much as 2 times. The ethanol extract obtained was then filtered using a Buchner funnel, then evaporated using a rotary evaporator, and concentrated using a waterbath.

Prior to fractionation, the optimization of the mobile phase is carried out to determine the comparative mobile phase that can separate the compound well. The mobile phase used is n-hexane and acetone with various comparisons (5 : 5, 7 : 3, and 9 : 1). The best separation is shown in the 7 : 3 ratio. Fractionation was performed using vacuum liquid chromatography. A total of 20 grams of ethanol extract were dissolved with 25 mL of 96% ethanol. After dissolving, 40 gram of silica gel 60 is added, stirred to homogeneous and the entire silica gel 60 is covered by the extract. In cleared columns arranged on stative and clamps, insert the silica gel 60G as much as 179 grams, then closed using filter paper. The column was saturated using 100 mL n-hexane 2 times. Then put the mixture of extract and impregnant silica into the already saturated column, then closed using filter paper. The eluent used is n-hexane : acetone with a ratio of 9 : 1 (3x), 8 : 2 (3x), 7 : 3 (3x), 6 : 4 (3x), 5 : 5 (as much as 2x) and ethanol (as much as 2x), with a single-pour volume of 150 mL. The elution results were collected in a bottle, then TLC test was done to classify polar, semipolar and nonpolar fractions.

2.2. Cytotoxic Test

As much as 10 mg sample added 100 µL dimetil sulfoksida, dissolved with the help of vortex. After dissolving, Dulbecco's modified eagle media added up to 1000 µL and then homogenized using a sonicator. Substance solution made from stock solution and diluting

dilution to the concentration of the resulting sample of 1000; 500; 250; 125; and 62.5 µg / mL. From each sample with various concentrations taken 100 µL inserted into the well that previously contained cancer cells MCF7 then incubated for 24 hours then given the reagent MTT. After formazan crystals formed, SDS is added and left overnight and read absorbance using ELISA reader. From the absorbance obtained then calculated the percentage of living cells by the formula:

$$\text{Percentage of living cells: } \frac{(\text{Control cell absorbance} - \text{Control media absorbance})}{(\text{Control solvent absorbance} - \text{Control media absorbance})} \times 100\%.$$

The relationship between concentration log and live cell percentage is shown in graphical form. The value of IC_{50} is determined from the linear regression equation (Y = BX + A), with the value of Y being 50%.

2.3. Test of Compound Content

The gembili herb extract which has low IC_{50} value is the semipolar fraction taken as much as 10mg then dissolved in 1mL of ethanol. The sample solution was bottled on the silica plate GF_{254}, then eluted with a n-hexane and acetone (7 : 3) as a mobile phase. The eluted and dried GF_{254} silica plate, observed under visible light, UV 254 nm and 366 nm UV. Then sprayed with several reagents such as Dragendorff to detect alkaloids, anisaldehid-H_2SO_4 to determine terpenoid content and saponins, sitroborates to detect flavonoids, $FeCl_3$ to detect phenolics, and re-observed under visible light and 366 nm UV.

3. THE RESEARCH RESULTS AND DISCUSSION

Immersion is the simplest method of sifting and is often an option because it does not have much physical disturbance and produces a high yield. The solvent used is 96% ethanol which is the primary preferred solvent for extracting secondary metabolites unknown to the structure. Ethanol 96% has an extensive canopy of power so that all secondary metabolites can be searched (Saifudin, 2014). The yield of ethanol extract obtained was 6.26%.

Fractionation is done by using vacuum liquid chromatography. Fractionation is an effort to separate chemical compounds based on the polarity level. Fraction classification is based on a high equation of Rf value or similar elution pattern. The higher the elution is, so it indicates that the compound is increasingly nonpolar. Based on Figure 1, the numbers 3, 4, and 5 are grouped into nonpolar fractions, 6 and 7 are semipolar fractions, and 8, 9 and 10 are polar fractions because they have the lowest Rf values.

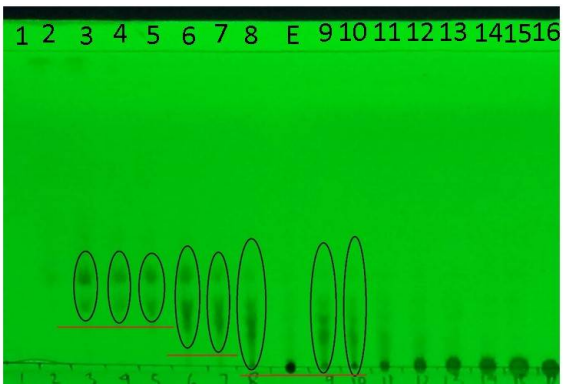

Figure 1. Chromatogram of the fractionated product on UV 254 nm.

The cells used in the cytotoxic test are MCF7 cells. The living MCF7 cells have an irregularly spherical shape, and have a clear color on the outside of the cell as shown in Figure 2 (A). In cells treated with 500 µg/mL, the semipolar fraction showed a change of the cells as small and black as Figure 2 (B) indicating the cell is dead, whereas in Figure 2 (C) is a MCF-7 cell that reacts with formazan. The living MCF-7 cells will reduce the formazan salt shown by the formation of purple (Riss *et al.*, 2013). Crystalline formazan is not water soluble it is necessary to add 10% SDS reagent in HCl 0.01 N to stop the reaction and dissolve it, so readable absorbance using ELISA reader. The higher the percentage of living cells, the purple color that is formed will be more concentrated, and the value of absorbance that is formed will be higher (Haryoto, 2013).

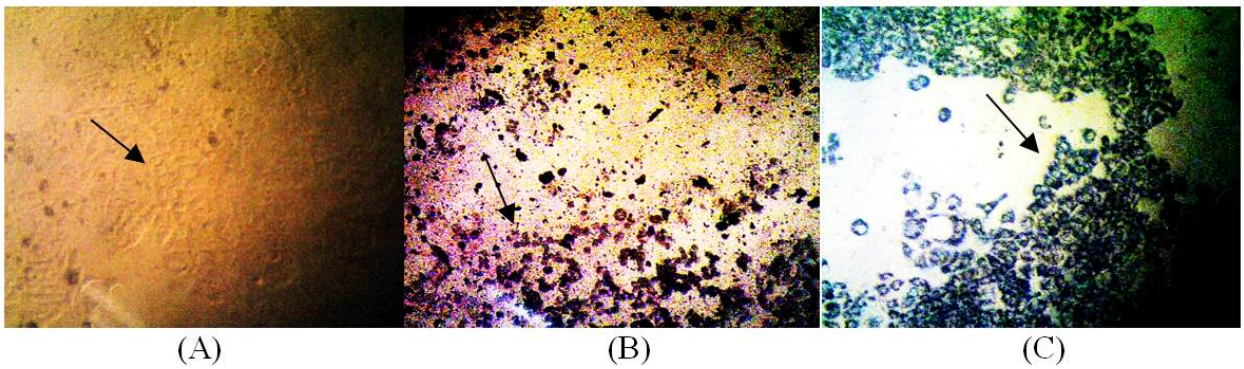

Figure 2. MCF-7 cell control (A), MCF-7 cells that died by treatment of 500 µg/mL semipolar fraction (B), and cells forming formazan crystals (C).

Calculation of IC50 values using linear regression between concentration and live cell percentage taken from 3 point concentration, that is 250, 500, and 1000 µg / mL. From Figure 3, it is known that the linear regression value of the semipolar fraction of gembili herb is y = -129.15x + 411,75 with linearity of 0.9828 which indicates that there is a correlation between concentration and the number of living cells, ie the higher concentration of samples given treatment then the number of living cells decreases.

National Cancer Institute states that an extract is said to have potential as an anticancer if it has an IC_{50} value <20 µg/mL (Bézivin *et al.*, 2003). Based on the results of the data

processing as shown in Table 1, the semipolar fraction of gembili herb has lower IC_{50} than the ethanol extract and other fractions, but much higher than the NCI standard of 632.42 μg/mL. Ethanol extract and polar fraction have IC_{50}>1000 μg/mL, whereas the nonpolar fraction of gembili herb has high live cell percentage so that its IC_{50} value can not be calculated.

Previous research mentioned that ethanol extract of gembili bulb has cytotoxic activity against T47D cell with IC_{50} value 39,61 μg/mL (Soetoko dan Sumarno, 2012). According to Muzaimah *et al*. (2010), methanol extract of *Dioscorea esculenta* L. leaves has IC_{50} 111,8 mg/L on Vero cell. The IC_{50} value of the semipolar fraction of the ethanol extract of gembili herb in this study is higher than the previous study could be due to different plant parts and cell types used. In addition, different plant-taking locations may also affect the outcomes.

Table 1. Calculation of % MCF7 cells that lived after treated and IC_{50} values of ethanol extract, nonpolar fraction, semipolar, and polar gembili herb.

Sample	Concentration (μg/mL)	Log concentration	Average of % live cell	Linear regression equation	IC_{50} (μg/mL)
Ethanol extract	250	2,398	141,065	y = -81,292x + 339,78	≥ 1000
	500	2,699	127,936		
	1000	3,000	92,123		
Polar fraction	250	2,398	117,068	y = -89,409x + 342,26	≥ 1000
	500	2,699	122,538		
	1000	3,000	63,239		
Semi polar fraction	250	2,398	105,033	y = -129,15x + 411,75	632,42
	500	2,699	57,257		
	1000	3,000	27,279		
Nonpolar fraction	250	2,398	133,771	y = 23,867x + 79,106	∞
	500	2,699	148,651		
	1000	3,000	148,140		

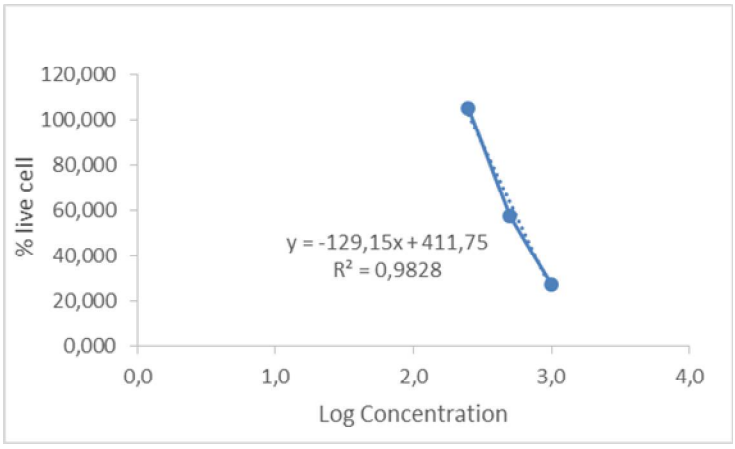

Figure 3. Linear regression between log concentration and percentage of MCF7 cells living at the semipolar fraction to calculate IC_{50} values.

Semipolar fraction has the lowest IC_{50} value compared to ethanol extract, nonpolar fraction, and polar, so it tested the content of the compound. The TLC profile of the semipolar fraction of the gembili herb can be seen in Figure 4. The spray reagents used to visualize the compound content include anisaldehyde-H_2SO_4, Dragendorff, $FeCl_3$, and sitroborate. TLC plates that have been sprayed with anisaldehyde-H_2SO_4 show a blue violet color so that positive contains terpenoids and saponins at Rf 0.45 and 0.65. The semipolar fraction contains alkaloids because of the brownish orange color after being sprayed with Dragendorff reagents with Rf values of 0.3 and 0.38 (Wagner dan Bladt, 2001). In addition, a bluish gray color appears after sprayed $FeCl_3$ reagent indicating that there is a phenolic compound in the sample with a value of Rf 0.38. The result of spraying of sitroborate reagents did not show any greenish yellow color so that the semipolar fraction of gembili herb did not contain flavonoid compound.

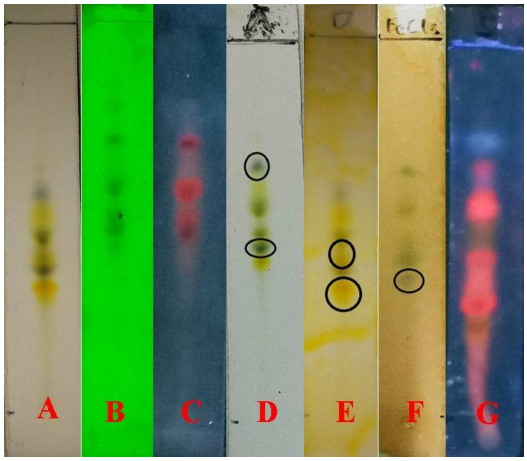

Figure 4. The TLS profile of the semipolar fraction gembili herb on visible light before being spray-reacted (A), on the UV 254 nm (B), on the UV 366 nm (C), after being treated with anisaldehyde-H_2SO_4 spray reagent in visible light (D), after being treated with Dragendorff spray reagent in visible light (E), after being treated with $FeCl_3$ spray reagent in visible light (F), and after being treated with sitroborate spray reagent in UV 366 nm (G).

Table 2. The results of detection of compounds contained in the semipolar fraction of herbal gembili.

No	Detection	Spotting	Rf	Color	Interpretation of Contained Compounds
D	Anisaldehyde-H_2SO_4	1 and 2	0,45 and 0,65	Blue violet	Terpenoid dan saponin
E	Dragendorff	3 and 4	0,3 and 0,38	Brownish orange	Alkaloid
F	$FeCl_3$	5	0,38	Bluish gray	Fenolic
G	Sitroborate	-	-	-	-

The previous studies have suggested that the gembili contains saponins, β-cystosterol, stigmasterol, cardiac glycosides, fats, starches, and diosgenin (Olayemi dan Ajaiyeoba, 2007). According to Muzaimah *et al*. (2010), gembili leaves positive contain saponins, but do not contain alkaloids and steroids or triterpenes. In research conducted by Soetoko dan Sumarno (2012) mentioned that the gembili bulb has a content of saponins such as diosgenin which has the potential as an anticancer. In this study, the semipolar fraction of gembili herb contains terpenoid compounds, saponins, alkaloids, and phenolics. The difference of compound content in this study with previous research can be caused by geographical location difference from the growth of research sample.

4. THE CONCLUSION

The chemical compounds contained in the semipolar fraction of gembili herbs are terpenoids, saponins, alkaloids, and phenolics. Based on these conclusions, it is recommended to conduct research on extracts and extracts of the gembili herb from other types of cancer cells to determine their cytotoxic activity.

5. ACKNOWLEDGEMENTS

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