CYTOTOXICITY OF METHANOL LEAF EXTRACT OF *ARTOCARPUS ALTILIS*, *ARTOCARPUS HETEROPHYLLUS*, AND *ARTOCARPUS CAMANSI* AGAINST MCF-7 BREAST CANCER CELLS

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ABSTRACT

Artocarpus had been used as traditional medicine for cancer, cirrhosis, malaria, hypertension, and diabetes. This research aimed to evaluate the cytotoxic activity of methanol leaf extract of *Artocarpus altilis*, *Artocarpus heterophyllus*, and *Artocarpus camansi* against MCF-7 breast cancer cell. The leaf extracts of *Artocarpus altilis*, *Artocarpus heterophyllus*, and *Artocarpus camansi* were obtained by the method of maceration with methanol. Phytochemical screening was performed by Thin Layer Chromatography with the use of silica GF-254 for stationary phase and ethyl acetate: n-hexane (2:8) for mobile phase. The cytotoxic test was conducted by MTT with diverse concentrations of extract, namely 500, 250, 125, 62.5, and 31.25 µg/mL. The results of cytotoxic test were measured by ELISA reader. Based on phytochemical screening, tannin, and alkaloids were found in three extracts. Cytotoxic test demonstrated that those extracts had low cytotoxic activity against MCF-7 breast cancer cells, with IC_{50} of 120.85; 339.46 and 530.88 µg/mL, respectively, for *Artocarpus altilis*, *Artocarpus heterophyllus*, and *Artocarpus camansi*.

Keywords*: Extract, TLC, MCF-7, IC50, cytotoxic, MTT assay*

INTRODUCTION

Cancer is the second leading cause of death globally with a percentage of 13% following cardiovascular disease (MoH, 2014). In 2012, the International Agency for Research on Cancer (IARC) reported breast cancer as the cancer with the highest percentage of new cases, which amounted to 43.3% with the percentage of death of 12.9% (Ministry of Health, 2015). Breast cancer treatment with chemotherapy is an option that has been chosen by the cancer patients in Indonesia. However, the treatment of cancer using chemotherapeutic agents may result in failure that causes drug resistance cancer cells (Stærk *et al*., 2002). Exploration of herbal ingredients is an effort to find anticancer chemotherapeutic agents that have high cytotoxic power and low side effects at the same time.

Some of plants that can be developed as an anticancer chemotherapeutic agent are included in the genus Artocarpus (*Artocarpus altilis*, *Artocarpus heterophyllus*, and *Artocarpus camansi*). Research has shown that the methanol leaf extract of breadfruit (*Artocarpus altilis*) contains a number of flavonoids geranyl dihydrochalcones, which have cytotoxic activity in a number of cancer cells, such as adenocarcinoma (SPC-A-1 cells), colon cancer (SW-480 cells), and hepatocellular cancer (SMMC-7721 cells) (Wang *et al*., 2007). Other studies mentioned the methanol extract of the wood of jackfruit (*Artocarpus heterophyllus*) has cytotoxic effects against B16 melanoma cells with IC_{50} of 10.3 µM and 12.5 µM at artocarpin and crudaflavone B (White *et al*., 2010). The cytotoxic activity of *Artocarpus camansi* has also been studied previously. The

ethanol leaf extract of *Artocarpus camansi* showed cytotoxic activity in colon cancer HCT116 cells with IC_{50} of 38.18 µg/mL (Tantengco & Jacinto, 2015). According to Saravanan *et al*., (2014) suggested several compounds of herbal ingredients, e.g. flavonoids, have been proven potential to be developed as anticancer chemotherapeutic agents, particularly in breast cancer.

Referring to previous studies, this research was conducted to determine the anticancer activity of the methanol leaf extract of breadfruit (*Artocarpus altilis*), jackfruit (*Artocarpus heterophyllus*) and breadnut (*Artocarpus camansi*) on MCF-7 breast cancer cells. It is expected the results of this study will provide a basis for the development of anticancer chemotherapeutic agents.

MATERIALS AND METHOD

Materials

Leaves of breadfruit, jackfruit, and breadnut, MCF-7 cells, the Dulbecco's modified eagle medium (DMEM), 10% FBS (Fetal Bovine Serum), 1% penicillin-streptomycin, 10% SDS (Sodium Dodecyl Sulfate), trypsin-EDTA (0.25%), DMSO (dimethyl sulfoxide), 0.5% Fungizone, a solution of MTT (*3-*(*4*,*5-dimethylthiazol-2-yl*)*-2*,*5-diphenyltetrazolium bromide),* PBS (Phosphate Buffered Saline), distilled water, methanol, 70% alcohol, spray reagent (FeCl3, sitroborat, and Dragendorff), GF-254 silica, n-hexane and ethyl acetate, liquid nitrogen tank, CO_2 incubator (Binder), inverted microscope (Olympus Japan), cell counters, vortex (Genie), a micropipette (Soccorex), blue tip and yellow tip (Greiner), a test tube, microtube, scales electrical, LAF (Nuaire), ELISA reader (ELX 800 Bio Tech), 96 well-plate (Iwaki), tissue culture flasks (Nunclon), tube conical sterile (Nunclon), hemocytometer (Marienfield Germany), sonicator, appliance glass (Pyrex), vacuum rotary evaporator (Heidolp), aluminum foil, a spatula, and a vacuum Buchner funnel and a digital camera (Sony).

Sample preparation

Samples of leaves of breadfruit, jackfruit, and breadnut were obtained from Sukoharjo, Central Java. Leaves were washed and aerated until the they were air dry and dried under the sun for 4-5 days to dry (bulbs). The simplicia was extracted and the yield was weighed.

Extraction

An amount of 200 grams of crude drug powder was soaked (macerated) using 750 mL of methanol for 24 hours, then filtered with a Buchner vacuum. The generated solution was evaporated into a thick extract. The dregs were re-macerated for 4 times with the same treatment. The evaporated yield was moved into a porcelain cup and placed on a water bath to evaporate the residual solvent and to obtain a thick extract.

Phytochemical screening

Optimization of mobile phase was carried out with 5 (five) ratios of (ethyl acetate: n-hexane), namely (5: 5), (4: 6), (3: 7), (2: 8) and (1: 9). Each extract was put on a TLC plate and stored in a chamber containing an optimum mobile phase and was saturated. Elution was performed with the optimum mobile phase of n-hexane: ethyl acetate (2:8). The plates were then sprayed to qualitatively assess the compounds with several reagents, including Dragendorff reagents to detect alkaloids, and tannins to detect FeCl3, and sitroborat to detect flavonoids (Saifudin, 2014).

Cytotoxic test

The cell suspension as much as 100 mL was put into the plate 96 and incubated for 24-48 hours in a 5% CO2 incubator. At the end of incubation, the media in each of the wells was removed, then added by 100 mL of sample in each of wells with variation of concentrations of 500, 250, 125, 62.5 and 31.25 mg/mL. Furthermore, the plate 96 was incubated in a 5% CO2 incubator for 48 hours at a temperature of 37ºC. At the end of incubation, the media in each of the wells disposed MTT was then added 100 mL of 5 mg/mL in PBS. Subsequently, Plate 96 was incubated for 2-4 hours in a 5% CO2 incubator at a temperature of 37ºC. Living cells would react with MTT (*3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide*) to form purple formazan. Formation of formazan reactions was stopped by the addition of 100 mL solution of 10% SDS (Sodium Dodecyl Sulphate) in 0.01N HCl into the respective wells, and then wrapped in paper and stored overnight at room temperature in a dark place. At the end of the incubation, absorbance was read with ELISA reader at a wavelength of 594 nm. The percentage of live cells was calculated by the absorbance of data cells, which was then made into curve of log concentration of living cells and % of values based on IC_{50}. Absorbance obtained from the ELISA reader was used to calculate the percentage of live cells with the formula:

$$\%\ Living\ cell = \frac{Absorbance\ treatment\ -\ Absorbance\ of\ media\ control}{Absorb.\ of\ solvent\ control - Absorb.\ of\ media\ control} \times 100\%$$

Subsequently, equation was made, which is y= Bx + A, with y=% live cells and x= log concentration (figure of log concentration versus the percentage of living cells and the calculated linear regression). The IC_{50} values were obtained by including y= 50 on the linear regression equation and the x was antilog, which was then calculated from these concentrations (CCRC, 2013). The IC_{50} value indicates the concentration which can lead to the death of 50% of the population.

RESULT AND DISCUSSION

Extraction

Results of bulbs with methanol extraction yielded 10.39% for jackfruit leaves, 11.85% for breadfruit leaves and 10.60% for breadnut leaves.

Phytochemical screening

The preliminary test of mobile phase indicated the optimal result of the ratio of ethyl acetate: n-hexane (2: 8). The stationary phase used for the analysis was silica GF-254, which is able to fluoresce at a wavelength of 254 nm. Phytochemical screening was performed to flavonoids, tannins and alkaloids.

The results of TLC analysis showed the methanol leaf extracts of breadfruit, jackfruit and breadnut contain tannins as detected by reagent FeCl3 spray (Figure 1e, 2e, 3e). Positive results were marked by colored patch of gray in the visible light (Saifuddin, 2014). The results are consistent with Pradhan *et al*. (2012) who reported tannin in the methanol leaf extract of breadfruit. Similarly, the findings reported by Burci Moura *et al*. (2015) also showed that the ethyl acetate extract of jackfruit contains tannin, which is in line with the results of this study since tannin is also identified in the phytochemical screening of the extract with methanol as the solvent. Results of the detection of reagent Dragendorff spray showed the methanol leaf extracts of breadfruit, jackfruit and breadnut positively contain alkaloids as indicated by brownish spots on visible light (Saifuddin, 2014). The results reaffirmed Khan *et al*. (2003) in

which the alkaloid compounds were produced by the methanol extract of leaves of jackfruit. Similarly, research conducted by Tehubijuluw and Southeast (2013) stated that the methanol extract of leaves of breadfruit contains alkaloids. Detection with the reagent sitroborat spray showed negative flavonoid in the methanol leaf extracts of breadfruit, jackfruit, and breadnut. A positive result is marked with yellow-green spots on the UV 366 nm (Markham, 1988). Apsari (2007) reported the presence of flavonoids in the methanol extract of bark of breadnut, thus the result opposes this study. It is due to the different parts of plant as the object of observation. Visualization of spots on TLC test (Table 1). Research conducted by Mohan *et al*., (2012) stated that the alkaloid compounds have activity as anticancer agents by inhibiting topoisomerase enzymes involved in DNA replication, induces apoptosis and p53 gene expression.

Cytotoxic test of leaf extract

Cytotoxic test conducted by dissolving the extract in DMSO. The DMSO level used in this test was 0.01% v/v. Theoretically, the use of high concentrations DMSO can cause the cell death. However, research by Jamalzadeh *et al*. (2016) suggested the use of 0.5% v/v DMSO does not affect the viability of MCF-7 cells. Observations of cell morphology showed insignificant difference between the control of MCF-7 cells and the control of DMSO treatments. The result of the methanol leaf extracts of breadfruit, jackfruit and breadnut generated the IC_{50} value of 120.85; 530.88 and 339.46 mg/mL, respectively (Table 2).

In theory, the relationship between the percentage of living cells and the concentration of the extract is an inversely proportional relationship. The lower the extract concentration, the higher the percentage of living cells (a dose-dependent phenomenon) (Khademvatan *et al*., 2016; Shiezadeh *et al*., 2013). The results of this study showed a dose-dependent phenomenon (Fig. 1) (Table 2).

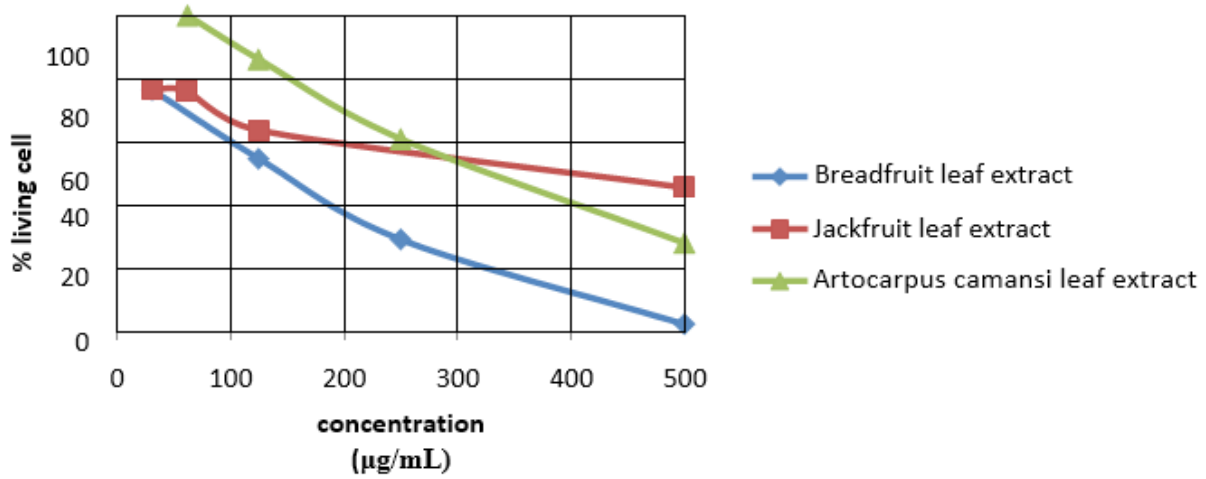

Fig. 1 Relationship between the concentrations of the methanol leaf extract of breadfruit, jackfruit and breadnut *(Artocarpus camansi)* and the percentage of living MCF-7 cells.

Table 1 Visualization of spots on TLC test

Leaf extract	The color appearance of patches						Result
	Before spraying			After spraying			
				Sitroborat	$FeCl_3$	Dragendorff	
	Visible light	UV 254nm	UV 366nm	UV 366nm	Visible light	Visible light	
Breadfruit	1 : yellow	1 : black	1 : reddish	1 : -	1 : grey	1 : -	Tannin, alkaloid
	2 : -	2 : yellow	2 : reddish	2 : reddish	2 : grey	2 : -	
	3 : yellow	3 : black	3 : -	3 : reddish	3 : -	3 : brown	
	4 : yellow	4 :	4 : reddish	4 : reddish	4 : -	4 : -	
	5 : black	5 : black	5 : black	5 : -	5 : -	5 : -	
Jackfruit	1 : yellow	1 : yellow	1 : reddish	1 : -	1 : grey	1 : -	Tannin, alkaloid
	2 : brown	2 : yellow	2 : reddish	2 : -	2 : grey	2 : -	
	3 : brown	3 : -	3 : -	3 : reddish	3 : -	3 : brown	
	4 : brown	4 : yellow	4 : reddish	4 : -	4 : -	4 : -	
	5 : black	5 : black	5 : black	5 : -	5 : -	5 : -	
Breadnut	1 : yellow	1 : yellow	1 : -	1 : -	1 : grey	1 : -	Tannin, alkaloid
	2 : -	2 : yellow	2 : reddish	2 : -	2 : grey	2 : brown	
	3 : yellow	3 : -	3 : reddish	3 : reddish	3 : -	3 : -	
	4 : yellow	4 : yellow	4 : reddish	4 : reddish	4 : -	4 : brown	
	5 : black	5 : black	5 : black	5 : -	5 : -	5 : -	

Table 2 Cytotoxic test data on the extracts of breadfruit, jackfruit, and breadnut

Leaf extract	Extract concentration (µg/mL)	Log concentration	% average living cell	Linear regression equation	IC_{50} (µg/mL)
Breadfruit	500	2.698970004	5.6331878	y = -66.01 + 190.3 r^2 = 0.93	120.85
	250	2.397940009	32.882096		
	125	2.096910013	58.820961		
	31.25	1.49850022	80.829694		
Jackfruit	500	2.698970004	49.519651	y = -28.00 + 126.3 r^2 = 0.952	530.88
	125	2.096910013	67.860262		
	62.5	1.795880017	80.305677		
	31.25	1.49850022	81.091703		
Breadnut	500	2.698970004	31.70306	y = -81.20 + 255.5 r^2 = 0.969	339.46
	250	2.397940009	65.10917		
	125	2.096910013	90.65502		
	62.5	1.795880017	104.6725		

The calculations show that the methanol leaf extract of breadfruit has the highest cytotoxic activity in compared with those of jackfruit and breadnut. Based on the criteria of the United States National Cancer Institute, a substance is classified as having the potential cytotoxic effect if the IC_{50} value is ≤ 20 mg/mL. The results of the acquisition of IC_{50} values in this study indicate that the three extracts of species in the genus Artocarpus have relatively low cytotoxic activity against MCF-7 breast cancer cells in which the potential of the extract is breadfruit > breadnut > jackfruit. The results show dissimilarities with previous studies (Table 3).

Table 3 Cytotoxic test data on previous research

Plant species	Part of plant	Solvent	Types of cancer cells	Compound	IC_{50}	Reference
Breadfruit	Leaf	Methanol	SPC-A-1 (adenocarcinoma)	Geranyl dihydrochalcone	28.14 µM	Wang *et al.*, 2007
			SW-480 (colon)		34.62 µM	
			SMMC-7721 (hepatocellular)		49.86 µM	
	Tree bark	Diethyl ether	T47D	Artocarpin	6.19 µg/mL	Arung *et al.*, 2009
		Ethanol	H460 (lung)	Artocarpin	7.9 µg/mL	Di, 2013
Jackfruit	Leaf	Methanol	PC-3 (prostate)	Flavonoid	8.3 µM	Patel and Patel, 2011
			A549 (lung)		35.26 µg/mL	
Artocarpus camansi	Leaf	Ethanol	HCTI 16 (colon)	-	38.18 µg/mL	Tantengco and Jacinto, 2015
			MCF-7 (breast)		9.58 µg/mL	

Based on the data, the obtained IC_{50} values are significantly different from previous studies. It might be due to the different types of cancer cell used in cytotoxic test, in addition to the different parts of the plant and the different solvent extraction. The compounds observed in relation with their potential cytotoxic activity in previous studies were flavonoids and their derivatives. The compounds were not involved in this study, thus the results of the acquisition of IC_{50} were different.

CONCLUSION

Research on MCF-7 breast cancer cells was done in pertaining with the effects of cytotoxic using the methanol leaf extract of breadfruit (*Artocarpus altilis*), jackfruit (*Artocarpus heterophyllus*), and breadnut (*Artocarpus camansi*). It can be concluded that the methanol leaf extracts of breadfruit, jackfruit and breadnut have anticancer activity against MCF-7 breast cancer cells t with IC_{50} values are 120.85; 530.88 and 339.46 mg/mL, respectively, which can be classified as low potential. The compounds contained in the methanol leaf extracts of breadfruit, jackfruit and breadnut are alkaloids and tannins. To complement this research, the cytotoxic test of other species in the genus Artocarpus and the cytotoxic test on different types of cancer cells using other plant parts of the breadfruit, jackfruit, and breadnut, are required.

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