

## Selectivity Index of *Alpinia galanga* Extract and 1'-Acetoxychavicol Acetate on Cancer Cell Lines

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### Abstract

Previous research stated that galangal (*Alpinia galanga*) extract has a potential as cytotoxic agent with active compound of 1'-Acetoxychavicol Acetate (ACA). The objective of this study was to determine the selectivity of ethanol extract, ethyl acetate fraction, and methanol fraction of galangal, and ACA on cancer cell lines. Cytotoxic activity was carried out using the MTT method on T47D breast cancer, WiDr colon cancer, HeLa cervical cancer, and Vero normal cell lines. The results showed that galangal ethanol extract and its fractions had selectivity index equal to or less than 2 on cancer cells. Meanwhile, ACA had selectivity index more than 3 on T47D cell and HeLa cell. ACA showed a strong cytotoxic activity against cancer cells T47D, HeLa, and WiDr with IC<sub>50</sub> values of 3.14, 7.26, and 12.49 µg/ml, respectively. Based on data, it could be concluded that ACA was the most selective to inhibit T47D cell with a selectivity index of 6.6.

**Keywords:** 1'-Acetoxychavicol acetate, galangal (*Alpinia galanga*), selective index, cytotoxic

### INTRODUCTION

Cancer is a disease that causes high mortality in the world. In 2012, 8.2 million deaths have been caused by this disease (Pusat Data dan Informasi, 2015). Chemotherapy, surgery, radiation, and hormonal therapy are the number of common therapies for cancer patients; however, they require high costs as well as a number of side effects due to the low selectivity of therapy. Researchers therefore continue to conduct research to obtain more selective anticancer drugs. One of the potential ingredients to be developed as anti-cancer with better selectivity is herbal medicines. One source of herbal medicines that has been traditionally used for cur-

ing cancer is galangal (*Alpinia galanga*) (Kuntorini, 2005).

Zaeoung, *et al.*, (2005) stated that *Alpinia galanga* as an antioxidant and free radicals scavenger has cytotoxic activity against MCF7 (breast adeno-carcinoma) and LS174T (intestinal adeno-carcinoma) cells. Galangal extract at the dose of 225, 450, and 750 mg/kgBW/day could increase the apoptosis process and reduce proliferative activity in breast cancer cells (Hartono, 2009). Cytotoxic

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activity, described as IC<sub>50</sub> value, on HeLa cervical cancer cell line of galangal extract from three local markets were 13.26, 36.32, and >100 µg/mL in order. Meanwhile, *Alpinia galangal* extract (AGE) from Pasar Legi (Surakarta, Indonesia) on MCF7 and T47D breast cancer cell lines have IC<sub>50</sub> value of 15.80 and 12.50 µg/mL, respectively (Suhendi et al., 2017). Galangal contains several phenylpropanoid compounds, including 1'-acetoxychavicol acetate (ACA), 1'-acetoxyeugenol acetate, trans-p-coumaril diacetate, 1'hydroxyccapsol acetate, and trans-coumaryl alcohol (Matsuda, et al., 2005). ACA is the main composition of *Alpinia galanga* (Baradwaj, et al., 2017; Hasima, et al., 2010).

ACA has cytotoxic against various cancer cell lines such as A549 cancer cells (lung cancer), SNU638 (stomach cancer), HCT116 (colon cancer), HT1080 (fibrosarcoma), and HL60 (leukemia) with IC<sub>50</sub> values of 8.14, 1.27, 1.77, 1.20, and 2.39µg/mL, respectively (Nam, et al., 2005). Another research by Zeng, et al., (2015) revealed that ACA showed cytotoxic activity on HeLa (cervical cancer), A549 (lung cancer), HepG-2 (liver cancer) and SMMC-7721 (liver cancer) with IC50 values of 85.1, 64.44, 74.51, and 61.27µg/mL, respectively. This study was conducted to determine the selectivity of galangal extract and ACA in breast cancer cells (T47D), cervical cancer cells (HeLa) and colon cancer cells (WiDr) compared to normal cells (Vero). The finding would serve as a basic for the further development targeted on cytotoxic research.

## METHOD

Materials that were used in this study include evaporator (Heidolph), waterbath (Changzhou Nuohai XMTD-204), analytical balance (Sartorius), micro pipette (Soccorex), LAF (Nuaire), hemocytometer (Marienfield Germany), 96-well-plate (Iwaki), conical tube, ELISA reader (BioTek), incubator (Binder), microscope (Olympus), galangal rhizome (Laboratorium Balai Besar Penelitian dan Pengembangan Tanaman Obat dan Obat Tradisional, Tawangmangu, Indonesia ), Ace-

toxychavicol acetate (LKT Laboratories Inc.), Roswell Park Memorial Institute (RPMI 1640, Gibco), dimethyl sulfoxide (DMSO) (Merck), Fetal Bovine Serum 10% (FBS, Gibco), penicillin-streptomycin 1% (Gibco), trypsin (Gibco), sodium dodecyl sulfate (SDS, Gibco), MTT (Sigma), fungizone (Gibco). Cell lines (T47D, HeLa, and WiDr) were obtained from Laboratorium Balai Besar Penelitian dan Pengembangan Tanaman Obat dan Obat Tradisional Tawangmangu, Karanganyar, Indonesia (B2P2TOOT).

## Extraction and fractionation

Extraction was conducted by maceration process with ethanol 95% as solvent within 3 days. Liquid extract was then evaporated to obtain the thick extract. Ten milligrams of thick extract were dissolved in 10 mL of distilled water and 10 mL of ethyl acetate in a separating funnel. The top layer (ethyl acetate partition) was taken and evaporated in a water bath covered in aluminum foil to form the ethyl acetate fraction. Subsequently, the ethyl acetate fraction was dissolved using methanol (methanol fraction). The fractionation was done in triplicates.

## MTT Assay

The MTT reagent (0.5 mg/mL) was prepared by taking 1 mL of stock solution of MTT in PBS (50 mg/10 mL) and diluted it by media up to 10 mL (for 1 well plate). Once disposed, the cell was washed with PBS and 100 µL MTT reagent was added to each well, including media control (without cells). The cell, following this, was incubated for 2-4 hours in a CO<sub>2</sub> incubator. The cell was then examined with an inverted microscope. After formazan was clearly formed, a stopper reagent (SDS 10%) was added in 0.1 N HCl. The plate was wrapped with paper or aluminum foil and incubated in a dark place at room temperature for one night. Absorbance of each well was then read by an ELISA reader at  $\lambda = 595$  nm. IC<sub>50</sub> was calculated based on liner regression equation between viability cells and concentration of samples (Mosmann, 1983).

### Selectivity Index Analysis

Selectivity Index (SI) is obtained from the  $IC_{50}$  value of a compound against normal cells divided by the  $IC_{50}$  value of cancer cells (Aljewari, *et al.*, 2010; Badisa, *et al.*, 2006). Compounds are classified as high selectivity if the SI value is  $>3$  and less selective if the SI value is  $<3$  (Sutejo, *et al.*, 2016).

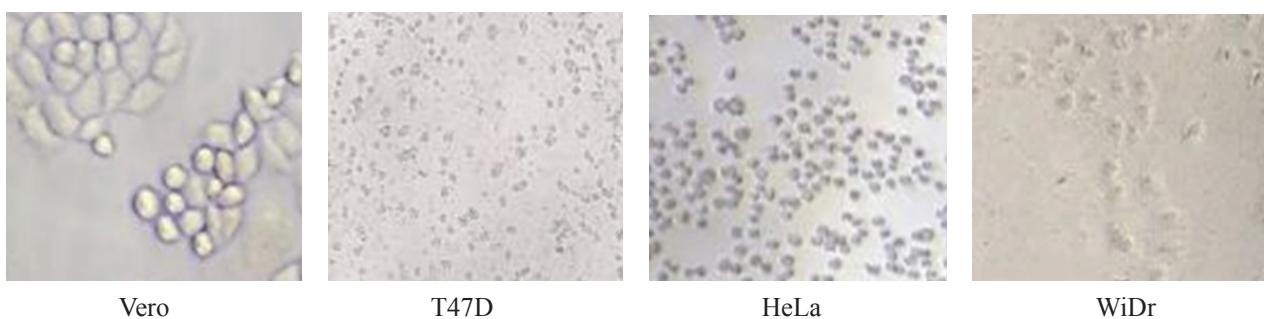
### RESULTS AND DISCUSSION

The material used in this study is galangal rhizomes obtained from the Center for Research and Development of Traditional Medicinal Plants and Medicines (B2P2TOOT) Tawangmangu, Karanganyar Regency, Central Java. Morphologically, the fresh galangal rhizomes are characterized with small and thick, fleshy, cylindrical about 2-4 cm in diameter, and branched. The outer parts are rather brown, reddish or pale greenish yellow with white and reddish, hard glossy scales, and while the inside part is white. The flesh of old rhizomes is rough fibrous. When getting dried, the rhizomes turn somewhat greenish, and the fibers become hard and tough. Dried rhizome was then extracted by maceration using 96% ethanol as solvent. The result of thick extract was 73.09 g with yield of 8.12%.

To group chemical constituents in extract, fractionation was conducted. The method used in fractionation of galangal extract was a liquid-liquid extraction. Grouping chemical constituent was based on distribution constants of chemicals in non-immiscible solvent (Berthod and Carda-Broch, 2004). The target of fractionation was to obtain the rich fraction of active constituent. One of active compounds of galangal extract is ACA, which has potential as an anticancer (Asri and Winarko, 2016). Fractionation results showed the yield of ethyl acetate fraction obtained as 7.43 gram, higher than the methanol fraction as 1.30 gram.

The cytotoxicity determination of galangal ethanol extract, ethyl acetate fraction, methanol fraction and ACA compounds were carried out using the MTT method on breast cancer cells (T47D), cervical cancer cells (HeLa), colon cancer cells (WiDr), and normal cells (Vero). Morphology of cancer cells after treated by samples is shown in Figure 1.

The value of  $IC_{50}$  of samples were calculated based on regression equation (Figure 2) of % viability cell percentage vs concentration. The value of  $IC_{50}$  of samples on three cancer cells showed a strong cytotoxic activity (Table 1). Due to  $IC_{50}$  values, the ACA is viewed to have the most active cytotoxic activity against all cancer cells tested. Ethyl acetate fraction has better cytotoxic activity compared to ethanol extract and methanol fraction. As ACA compounds are semi-polar, they could dissolve more in ethyl acetate. The compounds have the most active cytotoxic activity in T47D cells followed by HeLa cells and WiDr cells.  $IC_{50}$  values of



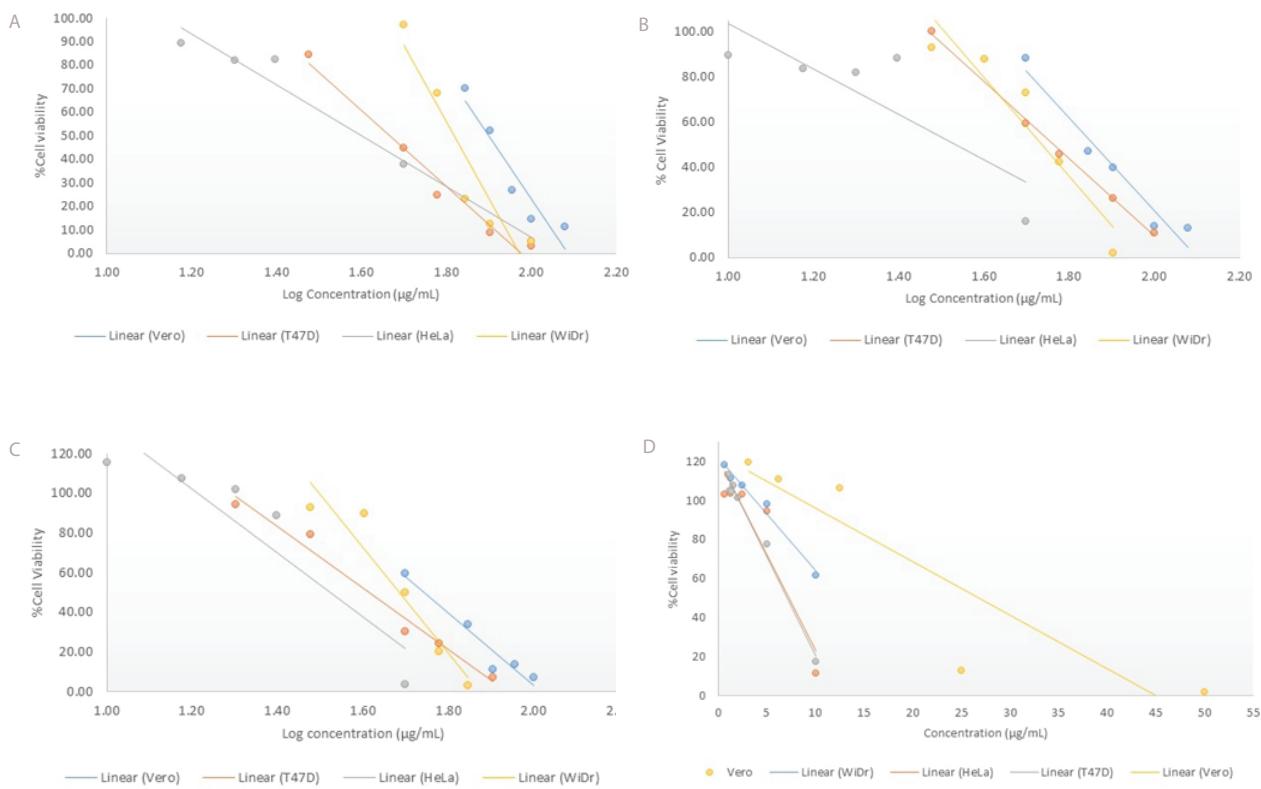
**Figure 1. Morphology of cancer cells after administration of ACA at a dose of 10 $\mu$ g/mL.** The cells were observed after 24 h of treatments under an inverted microscope with magnification of 100x.

**Table 1. Selectivity index of extract, ethyl acetate, methanol fractions and ACA on T47D, HeLa, WiDr, and Vero cell lines.**

Samples	T47D		HeLa		WiDr		Vero	
	IC <sub>50</sub> (µg/mL)	SI	IC <sub>50</sub> (µg/mL)	SI	IC <sub>50</sub> (µg/mL)	SI	IC <sub>50</sub> (µg/mL)	SI
Ethanol extract	44.93 ± 1.08	1.6	40.00 ± 0.55	1.9	66.11 ± 3.15	1.1	74.06 ± 7.13	1
Ethyl acetate fraction	42.29 ± 3.28	1.3	35.54 ± 0.44	1.5	48.81 ± 1.68	1.1	53.66 ± 2.57	1
Methanol fraction	40.49 ± 2.13	1.8	35.76 ± 1.53	2	55.24 ± 1.62	1.3	72.77 ± 0.35	1
ACA	3.14 ± 0.14	6.6	7.26 ± 0.12	3.5	12.49 ± 1.09	2	25.25 ± 0.92	1

ACA in T47D, HeLa, and WiDr were found in 3.14; 7.26, and 12.49 µg/mL, respectively. The potential of a compound is classified as strong cytotoxic agent if the IC<sub>50</sub> value <20 µg/mL, moderate if the

IC<sub>50</sub> value <50 µg/mL, and iweak if the IC<sub>50</sub> value > 50 µg/mL (Ellithey, et al., 2014) (Table 1). From the results obtained, it can be concluded that ACA has strong cytotoxic properties.



**Figure 2. Graphics of correlation of viability cell percentage vs concentration of ACA, ethanol extract of *Alpinia galangal*, and its fractions.** Percentages of viable cells were obtained based on colorimetric reaction of MTT that reduced by reductase enzyme resulting formazan and the absorbance was measured on wavelength 550 nm. Ethanolic extract showed negative slope that indicated the cytotoxic effect and ACA most potent to inhibit the cell growth on Vero, T47D, HeLa, and WiDr cell lines. A: ethanol extract of *Alpinia galangal*; B: methanol fraction *Alpinia galangal*; C: ethyl acetate fraction *Alpinia galangal*; D: 1'-acetoxychavicol acetate (ACA).

The selectivity of chemopreventive agents means that only cells identified as cancer cells are attacked. The higher the selectivity index of a compound to cells, the more selective the compounds to kill or inhibit the growth of a cancer cell with the smaller effect on normal cells. The small selectivity index indicates that the compound is less toxic to normal cells rather than to cancer cells. Compounds with high SI values offer the potential for safer and more effective therapy in cancer therapy (Segun, *et al.*, 2019). Based on the test results galangal ethanol extract, ethyl acetate fraction, and methanol fraction are found to be less selective for all tested cells ( $SI < 3$ ). Meanwhile, ACA is selective for T47D and HeLa cells with  $SI > 3$  and less selective for WiDr cells. The results of this research need a further study to determine the anticancer potential in animal models. Due to the lack of selectivity and many side effects (such as fatigue, nausea, alopecia and others) of cancer chemotherapy, these finding promising to develop as selective anticancer agent (González-Arriagada, *et al.*, 2013; Ihbe-Heffinger, *et al.*, 2013).

## CONCLUSION

The extract and fractions of *Alpinia galanga* ethanol extract have potential cytotoxic activity on T47D, HeLa and WiDr. Compound 1'-acetoxychavicol acetate has a strong cytotoxic activity against cancer cells T47D, HeLa and WiDr with IC<sub>50</sub> values of 3.14, 7.26 and 12.49 $\mu$ g/ml, respectively and selective for T47D breast cancer cells with selectivity index of 6.6.

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