

## Ethanollic Extract of *Arcangelisia flava* Leaves is Cytotoxic and Selective against Breast and Colon Cancer Cell Lines

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### ABSTRACT

Previous study revealed that ethanolic extract of *Arcangelisia flava* leaves (EEAFL) was able to increase immune response on doxorubicin-treated rats with no signs of toxicity. Its use as cancer co-chemotherapeutic agent could be based on its capability to decrease chemotherapeutic agent side effects as well as increasing the effectivity of chemotherapeutic agent. This study was determined to test the cytotoxicity and selectivity of EEAFL againsts several cancer cell lines as the basis for increasing the effectivity of chemotherapeutic agent indicator. The cytotoxicity assay was done by MTT method on HeLa, MCF-7, and WiDr cell lines as well as Vero cell line for the selectivity assay. EEAFL exhibited cytotoxic activity on HeLa, MCF-7, WiDr, and Vero with IC<sub>50</sub> value of 467+70; 136+17; 213+79; and 1340+288 ppm, respectively. Thus it was selective on HeLa, MCF-7, and WiDr with SI value of 2.87; 9.85; and 6.29, respectively. Meaning that EEAFL is cytotoxic and selective against MCF-7 and WiDr, but not on HeLa cell line. It can be concluded that EEAFL has the chance to be developed as cancer co-chemotherapeutic agent especially for breast and colon cancer, still there are ways to go.

**Key words:** breast cancer, colon cancer, cytotoxic, ethanolic extract of *Arcangelisia flava* leaves, selective.

### INTRODUCTION

Our previous studies revealed that ethanolic extract of *Arcangelisia flava* leaves (EEAFL) was able to increase the lymphocytes on doxorubicin-treated rats, suggesting that it increases immune response on doxorubicin-treated rats (Puspitasari and Umayah, 2013). It did not cause any signs of toxicity, biochemically nor histopathologically based on sub chronic toxicity assay (Puspitasari and Umayah, 2014). These results providing a prove that EEAFL could be used as cancer co-chemotherapeutic agent in combination with doxorubicin in order to decrease doxorubicin side effects.

Its use as cancer co-chemotherapeutic agent could be based on its capability to decrease chemotherapeutic agent side effects as well as increasing the effectivity of chemotherapeutic agent (Steward and Brown, 2013). This study was determined to test the cytotoxicity and selectivity of EEAFL againsts several cancer cell lines as the basis for increasing the effectivity of chemotherapeutic agent indicator. The cytotoxicity assay was evaluated based on the IC<sub>50</sub> obtained from MTT method (Doyle and Griffiths, 2000), while the selectivity was determined using selectivity index (SI) value (Prayong et al., 2008). This study was done on cervical, breast, and colon cancer cell lines, as well as on normal cell line for the selectivity assay.

## EXPERIMENTAL

### 2.1 Plant Extract Preparation

The *A. flava* leaves were collected from Meru Betiri National Park, Jember, Indonesia. They were selected for their freshness, old age, and healthy ones. The leaves were washed thoroughly with water, then, were air dried followed by oven drying at 50 °C. The dried leaves were grounded and sieved. The ethanolic extract were prepared using 100 g of leaves powder. The ground-dried leaves was extracted with ethanol 96%. The extraction was repeated three times. The ethanol extract was evaporated under reduced pressure (Heidolph, Laborota) resulting EEAfL. EEAfL was then suspended in DMSO never exceed than 1% for cytotoxicity assay.

### 2.2 Cytotoxicity Assay

The cytotoxicity assay was done by MTT method on several cancer cell lines. HeLa was representing cervical cancer cell line. MCF-7 was representing breast cancer cell line, while WiDr was representing colon cancer cell line. The normal cell was represented by Vero cell line. Those cell lines are the collection of Parasitology Laboratorium, Faculty of Medicine, Gadjah Mada University. The cytotoxicity assay was done for 24 hours, except HeLa cell line was treated for 48 hours. Briefly,  $1 \times 10^6$  cells were seeded in 96 well plate then incubated for 24 hours in 37 °C 5% CO<sub>2</sub> and suitable medium (HeLa was grown in DMEM low glucose, MCF-7 was grown in DMEM high glucose, WiDr and Vero were grown in RPMI) supplemented with 10% of fetal bovine serum and 1% of penicillin-streptomycin. A series of EEAfL then given to the cell line. At the determined time, the cells were washed with PBS and MTT (0.5 mg/ml) was added. The incubation was continued for 4 hours. Then the stopper reagent (10% SDS in 0.1 N HCl) was added and the absorbance was read at 595 nm. The cell viability was calculated as follows:

$$\text{Cell viability} = \frac{\text{Absorbance of treated cell} - \text{Absorbance of medium control}}{\text{Absorbance of cell control} - \text{Absorbance of medium control}} \times 100 \%$$

The IC<sub>50</sub> was determined by probit analysis based on the plot of concentration vs cell viability (Doyle and Griffiths, 2000).

### 2.3 Selectivity Assay

The selectivity index (SI) was determined based on the IC<sub>50</sub> values and calculated as follows:

$$\text{Selectivity index (SI)} = \frac{\text{IC}_{50} \text{ of normal cell line}}{\text{IC}_{50} \text{ of cancer cell line}}$$

(Prayong et al., 2008).

### 2.4 Statistical Analysis

The IC<sub>50</sub> were presented as mean + standard deviation (SD) from triplicate. Then they were analyzed using Anova followed by LSD (p 0.05).

## RESULTS AND DISCUSSION

The EEAfL obtained was 16.1 gram from 100 g ground-dried leaves. The yield was 16.1%. The cytotoxicity assay showed that the cell viability of all four cell lines were decreased in dose dependent manner when treated by EEAfL (Fig. 1). The IC<sub>50</sub> value of EEAfL on HeLa, MCF-7, WiDr, and Vero was 467±70; 136±17; 213±79; and 1340±288 ppm, respectively (Table 1). EEAfL was classified to have moderate cytotoxicity on HeLa, MCF-7, and WiDr cancer cell lines, but considered non toxic on normal cell line (Prayong et al., 2008). Based on the IC<sub>50</sub>, the EEAfL exhibited cytotoxic on HeLa, MCF-7, and WiDr cancer cell line, but not on normal cell (Vero).

The methanol extract of *A. flava* stem exhibited cytotoxic activity on MCF-7 cell line with IC<sub>50</sub> value of 7.7±0.6 ppm (Keawpradub et al., 2005). The distinct between these results may contributed by the active substance content in both extract. One of the active substance in *A. flava* known to have anticancer properties is berberine (Yu et al., 2007; Eom et al., 2008; Katiyar et al., 2009; Pandey et al., 2008; Kim et al., 2008). The EEAfL only contains 0.14% berberine (Puspitasari and Umayah, 2013), while berberine in methanol extract of *A. flava* stem contains 6.27% (Keawpradub et al., 2005). That is why the cytotoxic activity of methanol extract of *A. flava* stem is higher than EEAfL.

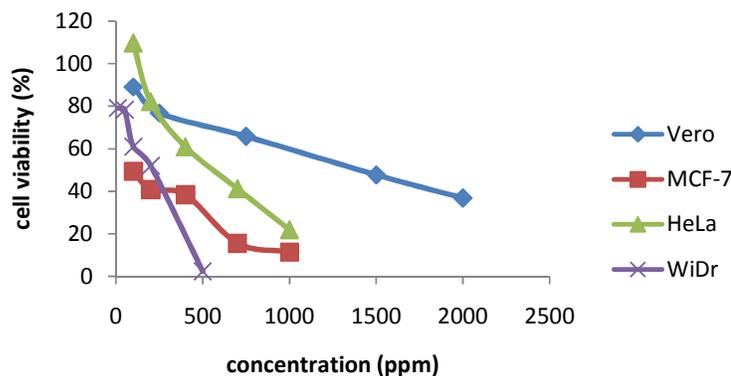


Fig 1. Cancer cell lines' viability due to EEAfL treatment for 24 hours (except HeLa cell line were treated for 48 hours).

The SI value of EEAfL on HeLa, MCF-7, and WiDr was 2.87; 9.85; and 6.29, respectively (Table 1). SI value more than 3 was classified to be high selective, while SI value less than 3 was classified to be less selective (Prayong et al., 2008). EEAfL was considered to have high selectivity on MCF-7 and WiDr, but less selectivity on HeLa cell line. The EEAfL could be taken for further bioassay guided experiment as anticancer against breast and colon cancer based on these findings, including the molecular mechanisms investigations and the possibility of the use in combination with cancer chemotherapeutic agents in order to obtain maximum effect and minimum side effects.

Table 1. IC<sub>50</sub> and SI of EEAfL on several cancer cell lines

No	Cell line	IC <sub>50</sub> (ppm)	SI
1	HeLa	467 + 70 <sup>a</sup>	2.87
2	MCF-7	136 + 17 <sup>b</sup>	9.85
3	WiDr	213 + 79 <sup>a,b</sup>	6.29
4	Vero	1340 + 288 <sup>c</sup>	

\*The IC<sub>50</sub> data was shown in mean + SD (n=3).

\*\*Different notation on IC<sub>50</sub> showed significant difference by LSD ( $p < 0.05$ )

## CONCLUSIONS

Based on the results, we can conclude that EEAfL is cytotoxic and selective against breast and colon cancer, but not on cervical cancer. It has the chance to be developed as cancer co-chemotherapeutic agent especially for breast and colon cancer, still there are ways to go.

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