

# Anticancer Activity of *Fibraurea Tinctoria* with DLD1 Celline Cytotoxicity Assay

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Abstract: This study aims to determine the cytotoxic effects of *Fibraurea tinctoria* plants on DLD1 colon cancer cell lines in vitro and determine the IC<sub>50</sub> values of these plants. The test begins with cell-line culture in the DMEM medium. Cultures that were ready with an estimated concentration in fresh media of 2.5x 10<sup>4</sup> cells / 200 $\mu$ L were exposed to 0.4 $\mu$ L test material in 0.1% DMSO with concentrations starting at 6.25 ppm; 12.5 ppm; 25 ppm; 50 ppm; 100 ppm; 200ppm; 400ppm; 800ppm; and 1600ppm which was then incubated for 48 hours. The results are from methanol extract, hexane fraction, ethyl acetate fraction and HA and HB subfraction from *Fibraurea tinctoria* plants, only HB subfraction which shows inhibitory activity against DLD1 colon cancer cell line with 28% at 1600 ppm concentration.

## 1 INTRODUCTION

The International Agency for Research on Cancer estimates the mortality and prevalence of cancer for 184 countries in the world at 14.1 million new cancer cases, with 8.2 million cancer deaths, and 32.6 million people living with cancer (within five years of diagnosis ) in 2012. By 2030, it is projected that there will be 26 million new cancer cases and 17 million cancer deaths per year (Solowey et al. 2014; Thun et al. 2010). Colorectal cancer is a disease that significantly attacks millions of people every year worldwide and is considered cancer with the third most common occurrence rate in men and number two in women. In the western world, with 655,000 deaths per year, colorectal cancer ranks third in cancer-causing deaths (Granlund et al. 2011; Selek et al. 2018).

Natural products have played a major role in cancer chemotherapy. Anti-cancer drugs were introduced into therapy in Western countries for around 70 years, around 49% were either obtained from direct organisms or derived from natural material products (Gurnani et al. 2014). Actinomycin D, several anthracycline D derivatives (including daunorubicin, doxorubicin, epirubicin, idarubicin,

and valrubicin), bleomycin, valcicin, cyclic and valrubicin derived from microbes that have been tested for cancer chemotherapy (Cragg, Grothaus, and Newman 2009) (Kinghorn et al. 2009). At present 4 ingredients that become anti-cancer drugs derived from plants are used clinically in the US and Europe, namely vinca (*Catharanthus*) bisindole alkaloids (vinblastin, vincristine, vinorelbine, vinflunine), semi-synthetic epipodophyllotoxins (etoposide, teniposide, and etoposide phosphate), taxel (paclitaxel and paclitaxel albumin-stable nanoparticle formulations, docetaxel, cabazitaxel), and campotekin derivatives (irinotecan and topotecan) (Kinghorn et al. 2016).

In Indonesia itself, the use of medicines from natural ingredients is a culture and hereditary habits. Kayu kuning is a plant that is used as an anti-cancer therapy and also in conditions of digestive tract infections. One type of yellow wood is tinctorial *Fibraurea* (Wahyudi, Ratnadewi, and Siswoyo 2016). This plant was reported to have inhibitory activity against MCF-7 cell line from chloroform extract with IC<sub>50</sub> value as significant as 11.2 ppm, although methanol and water extracts did not show significant activity to inhibit cell line extracted from breast cancer (Keawpradub and Dej-adisai 2005)

Due to the presence of empirical information about its ability to treat conditions in gastrointestinal infections, a search of the colonic cell line to see the potential of this plant as an agent for colon-rectal therapy.

## 2 METHODS

### Material

The research material in the form of *Fibraurea tinctorial* plants was obtained from the Wiranto Kadri Forest of Samboja East Kalimantan. Plant parts used are the stem. Parts of this plant are then dried, cut and mashed into simplicia powder.

### Extract, Fraction and Isolate Preparation

Simplisia powder was extracted by the reflux method. The extract obtained is then concentrated. The extract was subsequently fractionated using organic solvents with multi-level polarity. The obtained fractions were then separated by the classical column method to produce several nearly pure *Fibraurea tinctorial* isolates.

### Cancer Colon Cancer Cell Preparation DLD1

The DLD-1 cell line was obtained from the Biotechnology Center BPPT Serpong, Indonesia. Cells were grown in Dulbecco modified Eagle's medium (DMEM) with 4 mM L-glutamine and 10% heat-inactivated fetal calf serum. Cells were cultured at 37 ° C in a humidified atmosphere of 95% water

and 5% CO<sub>2</sub>, refed every two days and passaged weekly. Cells were allowed to grow for 72–96 hours to confluence before use.

### Test Material Preparation

Extracts, fractions and isolates of tinctorial *Fibraurea* plants were dissolved using 100% DMSO with a final concentration of DMSO of 1% on the test material. The UI material is placed in the microwell plate with the test material template located in the second column with dilution pointing to the right column up to column 11. The 12th column contains positive control of staurosporine, while column 1 contains 100% DMSO. All test materials are Duplo.

### Testing the Test Material against Cancer Colon Cancer Cells DL1

Count the cell number of remaining cell suspension from passage (Calculate average of cell number from 3 different squares, Calculate the concentration of cell suspension using formula below, concentration = (average of cell number from 3 different squares x 10<sup>4</sup> x dilution rate) cells/mL, Do not forget to multiple by the dilution rate). Prepare cell suspension with the following concentration using fresh medium (Cell number for DLD1 : 1.25 x 10<sup>5</sup> cells/mL (2.5x 10<sup>4</sup> cells/200mL)). Put 100 μL of the cell suspension to each well of 96 well plates. Place the plate in 37°C incubator for 24 hours (overnight). Add 0.4 μL of each extract (dissolved in 100% DMSO) for each well. Place the plate in incubator (37°C, 5% CO<sub>2</sub>) incubator for 48 hours. Typical 96-well plate layout for cytotoxicity assay showed figure 1.

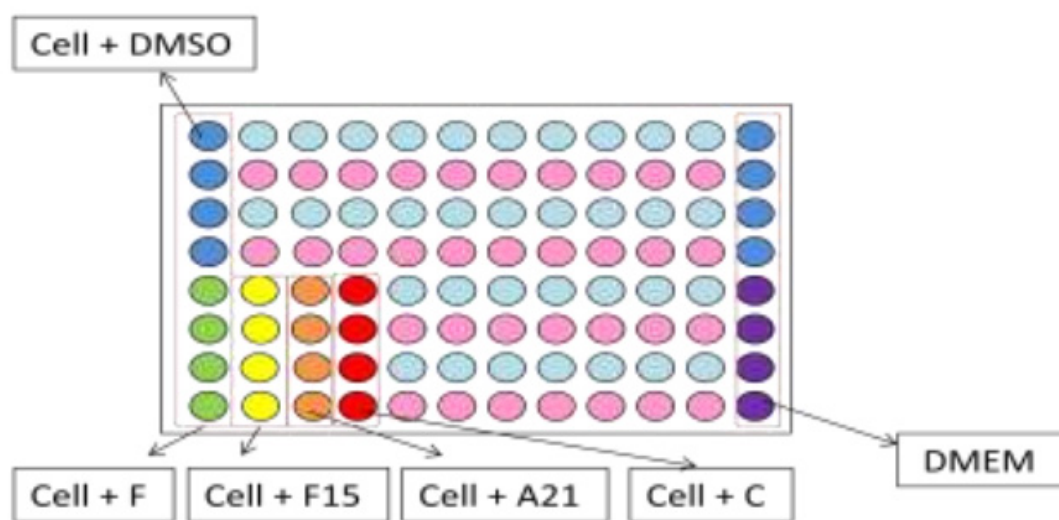


Figure 1: Typical 96-well plate layout for cytotoxicity assay

### Calculate Result

We have removed the medium from the plate by aspirator (using 1 mL serology pipette with yellow tip). Change the tip for each sample. Wash the cell by 100  $\mu$ L of PBS for each well. Aspirate the PBS. Add 1 mL of CCK-8 into the tray, then add 10 mL of DMEM medium on it. Mix well. Add 100  $\mu$ L of DMEM containing CCK-8 into each well. Place the plate in 37°C incubator for 3 hours. Measure the absorbance of each well at 450 nm by a plate reader. Calculate the survival rate as follow for each extract and their medium

$$\text{Survival rate}(\%) = \frac{(As - Ab)}{(Ac - Ab)} \times 100\% \quad (1)$$

As : Abs of sample well;

Ac : Abs of control well (DMSO);

Ab : Abs of Positive control well (Staurosporine)

## 3 RESULTS AND DISCUSSION

This test aims to determine the ability of *Fibraurea tinctoria* plants to inhibit dld1 cell line as a picture of

inhibition of rectal colon cancer that occurs in humans. The study began by collecting *Fibraurea tinctoria* plants, extracting and separating active compounds based on the polarity of the compounds contained in these plants.

Extracts, fractions and compounds of the separated product were tested on cell line dld1 culture in the DEMB media according to the procedure stated. The test material was presented with cell cultures in varying concentrations ranging from 6.25 ppm to 1600 ppm. The test results show the value of % survival rate, which can be seen in Table 1.

Seen from Table 1 above, the % survival rate of all test materials and concentration variations are at values above 90% or equal to DMSO (negative control) which means there is no specific concentration of extract, fraction or isolate material which results in inhibition of cell line dld1 growth. HB isolates showed inhibition to the percentage of cell lines that grew by only 24.48%, but this only occurred at a concentration of 1600 ppm. The value of % survival if presented in the form of a curve can be seen in Figure 1.

Tabel 1: Percentage survival rate *Fibraurea tinctoria* pada cell line dld1

Concentration (ppm)	Survival rate (%)				
	Isolat HA	Isolat HB	Hexan extract	Etilasetat extract	Methanol extract
1600.00	108.44 ± 0.030	24.48 ± 1.415	83.069 ± 0.017	99.58 ± 0.006	111.61 ± 0.034
800.00	90.89 ± 0.153	111.32 ± 0.009	95.92 ± 0.495	112.62 ± 0.001	110.93 ± 0.005
400.00	105.24 ± 0.012	110.16 ± 0.057	98.13 ± 0.149	107.867 ± 0.056	116.51 ± 0.065
200.00	107.27 ± 0.068	103.86 ± 0.166	90.55 ± 0.010	110.42 ± 0.125	110.27 ± 0.053
100.00	110.39 ± 0.085	109.42 ± 0.040	108.96 ± 0.032	105.99 ± 0.265	117.28 ± 0.041
50.00	111.87 ± 0.087	98.45 ± 0.995	94.14 ± 0.338	107.77 ± 0.003	115.22 ± 0.020
25.00	102.20 ± 0.100	107.49 ± 0.034	102.7 ± 0.245	110.93 ± 0.048	113.01 ± 0.08
12.50	104.17 ± 0.040	101.98 ± 0.077	90.80 ± 0.098	97.36 ± 0.048	107.84 ± 0.09
6.25	107.99 ± 0.012	102.99 ± 0.089	90.61 ± 0.015	104.00 ± 0.1	110.1 ± 0.040
3.13	110.97 ± 0.023	108.74 ± 0.120	81.76 ± 0.021	98.87 ± 0.13	109.16 ± 0.042
Positif control (staurosporin)			1.89 ± 0.82		
Negative control (DMSO)			-		

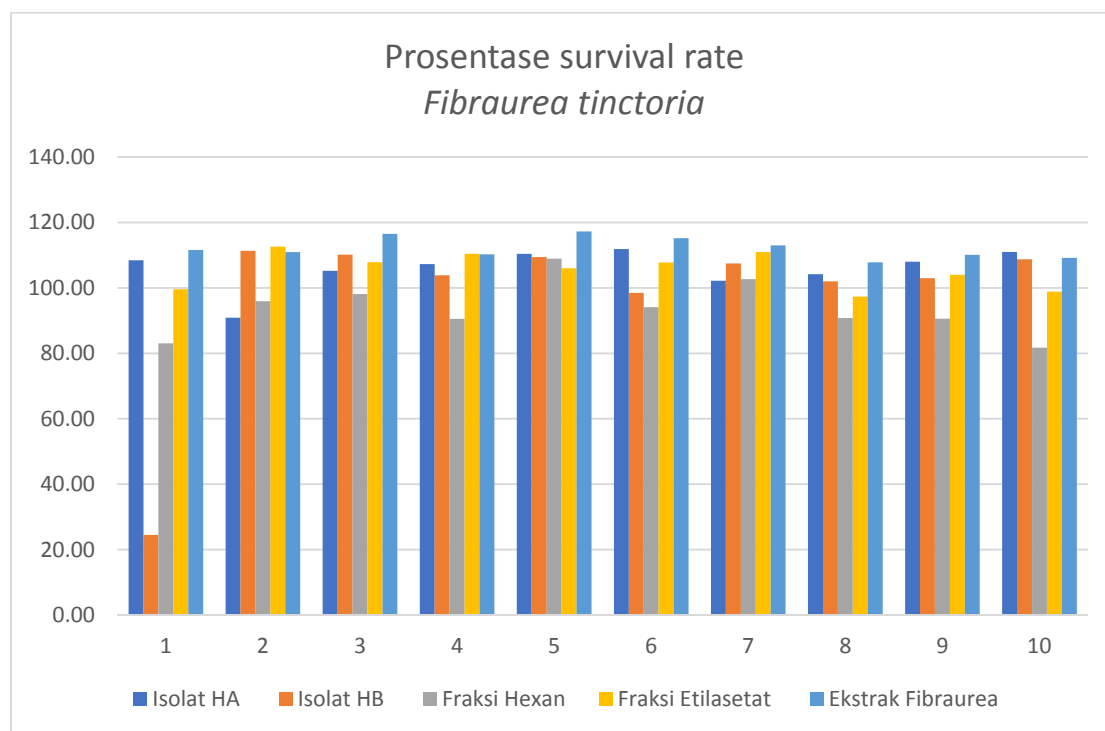


Figure 2: Chart of survival rate *Fibraurea tinctorial*

From the overall results of the test and the value of % survival rate, the IC<sub>50</sub> value is calculated by the linear regression equation, the IC<sub>50</sub> value obtained is shown in table 2 below:

Table 2: IC<sub>50</sub> of *Fibraurea tinctorial*.

Sample	IC <sub>50</sub> (ppm)
Isolat HA	> 100
Isolat HB	>100
Hexan extract	> 100
Etilasetat extract	> 100
Methanol extract	> 100

From the table above, if correlated with the literature which states that the extract has potential as an anticancer if the IC<sub>50</sub> value <30ppm (Istiqomah, Muti'ah, and Hayati 2015) and pure isolate if the IC<sub>50</sub> value is at a concentration of 2-4 ppm (Heliawati et al. 2015) then the entire material above has no potential as an anticancer in dld1 cell line cell culture. This test does not mean to be useless, but it can be information that if used as other medicinal ingredients, extracts, fractions and isolates from *Fibraurea tinctorial* plants have an extensive therapeutic range to no toxic effect on body cells both normal and abnormal (Cancer).

From this research, it can be concluded that the extract of methanol, n-hexane fraction, ethyl acetate fraction, and *Fibraurea tinctoria* isolate did not have anticancer activity with the testing model of dld1 cell line.

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