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# SELECTIVITY OF ETHANOL EXTRACT OF PARIJOTO *(MEDINILLA SPECIOSA)* FRUIT IN HEPG2, WIDR, 4T1, AND VERO CELL LINES

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**ABSTRACT**

**Background:** Parijoto, one of the melastomaceae family, has been known to have cytotoxic activity in some cancer cell lines, such as HeLa, MCF-7, and T47D. **Aims:** We aim to know about the selectivity of ethanol extract of Parijoto fruit in cell line HepG2, WiDr, 4T1, and Vero. **Method:** Extract was added in three serial concentration three serial concentrations (125 µg/mL–500 µg/mL), while the positive control doxorubicin gives in 2,5 µg/mL – 20 µg/mL for cancer cell and 40 µg/mL -100 µg/mL for Vero cell**. Results:** Results showed that ethanol extract of parijoto fruit gave low activity in HepG2 and Vero cell (IC50: 250 µg/mL) and moderate activity in WiDr and 4T1 (IC50: 81,58 µg/mL and 158,72 µg/mL). **Conclusion:** The highest selectivity index is given in WiDr cell (SI> 3) means that the ethanol extract of parijoto fruit is a promising cytotoxic agent for colorectal cancer therapy.

***Keyword:*** *Parijoto, Medinilla speciosa, HepG2, WiDr, 4T1, Vero*

## INTRODUCTION

Cancer is one of the mortality diseases in the world. Current research in developing cancer agents has been done by in vitro study on some plants with biologically active compounds. Parijoto is one of the medicinal plants for cytotoxic agents which is the Melastomaceae family. Melastomaceae is known to have some biologically active compounds such as, terpenoids, simple phenol, flavonoids, quinone, lignan , and their glicoside, tannin, or polyphenol[1].

Tussanti, et al., 2014 shown that ethanolic extract of parijoto fruit has cytotoxic activity with an IC50 value as 614.50 µg/ml[2]. Another research conducted that the methanolic extract of Parijoto has a synergistic effect with cisplatin against HeLa cell line[3]. Then, Annisa, et al., 2021 shown that fractions of ethanol extract gave cytotoxic activity in 4T1 cell line[4]. Another research conducted that ethanol extract gave IC50 as 178, 38 µg/ml ini HeLa cell line[5] and 121,56 ppm in MCF-7 cell line[6].

Our research study aims are search the selectivity of ethanol extract of parijoto fruit in HepG2, WiDr, T41, and Vero cell lines. The results has become the basis of developed parijoto fruit extract as cytotoxic agents.

## METHOD

### Preparation of Parijoto Fraction

*Medinilla speciosa* (Parijoto) ripe was taken from Muria Mountain, Colo Village, Kudus Regency, Central Java, Indonesia. Plant identification has been done by Biology Laboratorium, Sains and Mathematics Faculty, Universitas Diponegoro. *Medinilla speciosa* ripe was macerated by ethanol 70% (Brataco, Indonesia) in 3 days. The extract was evaporated by rotary evaporator at 40°C.

### Cytotoxic activity

Cell line ($1\times10^5$ cell/well) has been cultured in their medium on 96 well-plate and incubated in 24 hours. Sample (extract) was added in three serial concentrations (125 µg/mL–500 µg/mL), while the positive control doxorubicin gives in 2,5 µg/mL – 20 µg/mL for cancer cell and 40 µg/mL -100 µg/mL for Vero cell and incubated overnight. As much as 100 ul MTT (Sigma Aldrich) 5mg/mL on PBS was added onto each well and incubated for four hours until formazan cristal form. The reaction has been stopped by adding 100 ul SDS 10% and incubated overnight in light-protected conditions. The absorbance was determined in 545 nm by *ELISA reader*. Absorbance was formulated to calculate % cell viability by the following formula:

$$\frac{(cell\ control\ abs - medium\ abs) - (sample\ abs - medium\ abs)}{(cell\ control\ abs - medium\ abs)} x\ 100\%$$

The experiments were done by three times replication; then the IC50 value was taken from interpolated log concentration vs % cell viability by linear regression

### Selectivity Index Analysis

Selectivity Index (SI) is calculated from the IC50 value of a sample against normal cells divided

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by the IC50 value of cancer cells[7;8]. Samples are classified as high selectivity if the SI value is >2

## RESULTS

Cytotoxic activity can be shown by IC50 value that is obtained from linear regression of log C vs % cell viability (figure 1 and 2). The IC50 value is showed the sample ability to inhibit 50% cancer cell line proliferation. Then, the IC50 value of Vero cell is divided by IC50 cancer cell to obtained the selectivity index of ethanol extract of parijoto fruit and doxorubicin in HEPG2, WiDr, and Vero cell line (Table 1)

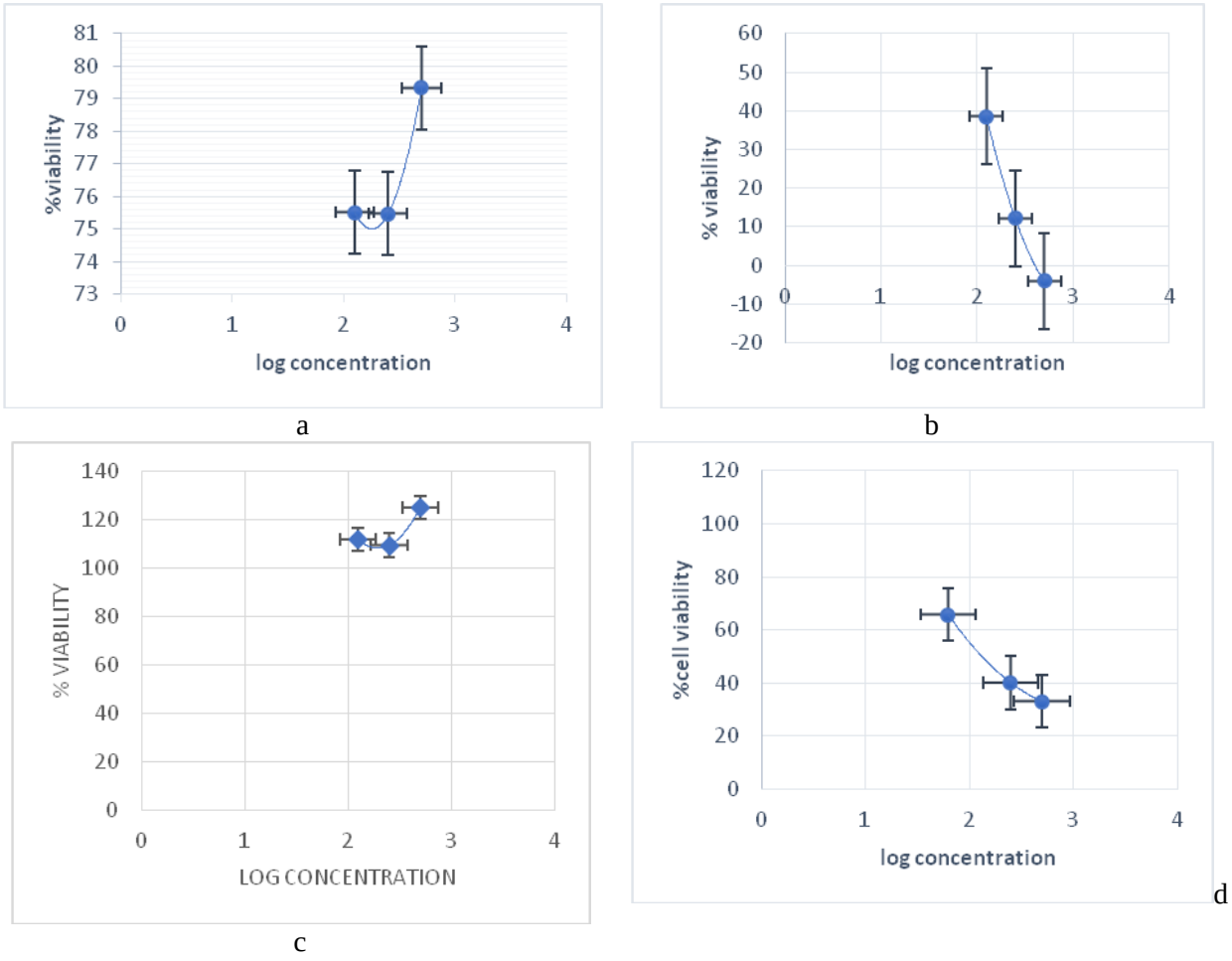


**Figure 1.** Cell viability after treatment by ethanol extract of parijoto fruit (500, 250, and 125 ug/ml). a. HepG2., b. WiDr., c. Vero., d. 4T1

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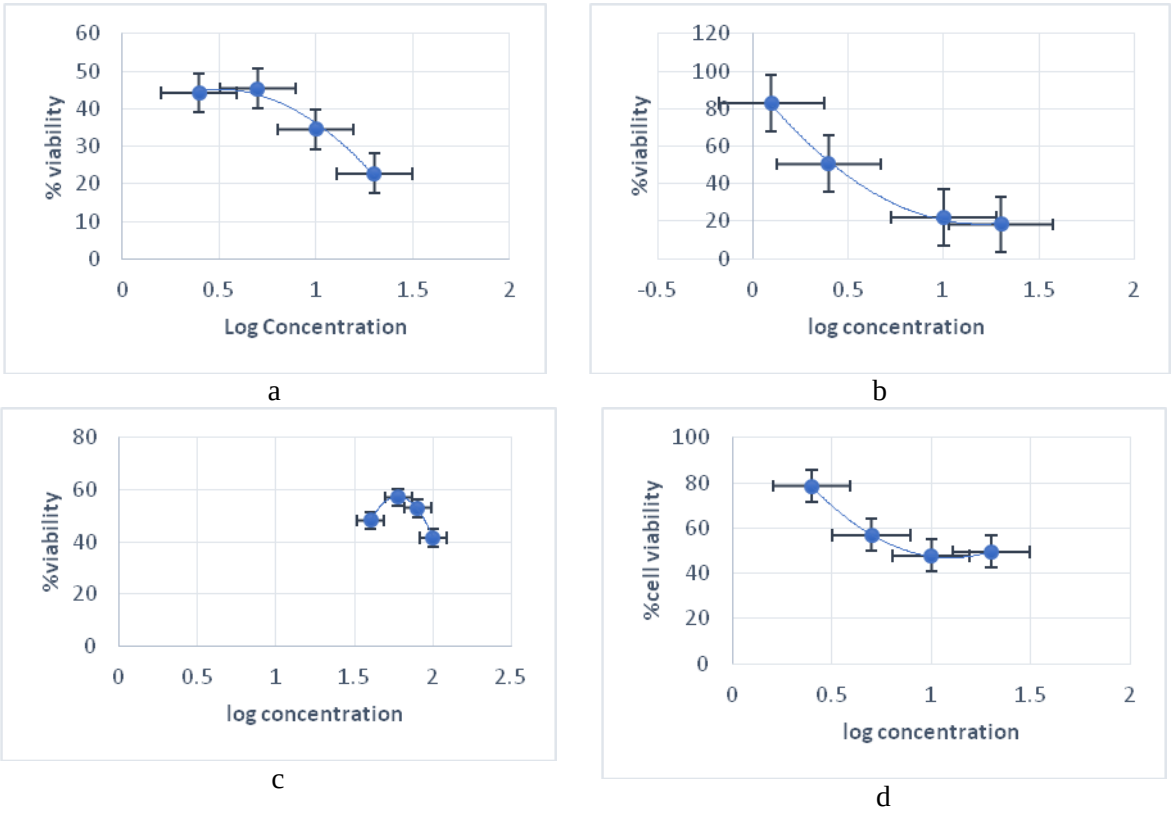


**Figure 2.** Cell viability after treatment by doxorubicin (20-1,25 ug/ml) for a cancer cell, and 100-40 ug/ml for Vero cell). a. HepG2., b. WiDr., c. Vero., d. 4T1

**Table 1.** IC50 value and selectivity index of ethanol extract of parijoto fruit and doxorubicin in HepG2, WiDr, and Vero cell line

| Sample | IC50 | | | | Selectivity index (SI) | | | |
|---|---|---|---|---|---|---|---|---|
| | HepG2 | WiDr | 4T1 | Vero | HepG2 | WiDr | 4T1 | Vero |
| Extract | 250* | 81,58 | 158,72 | 250* | 1 | 3,06 | 1,58 | 1 |
| Doxorubicin | 2,08 | 3,74 | 12,72 | 79,61 | 38,27 | 21,29 | 21,26 | 1 |

* IC50 value is obtained from the highest concentration that gave lowest % cell viability
IC50> 1000 ug/ml is written as not determined (ND)
SI was calculated by IC50 Vero cell / IC50 cancer cell

## DISCUSSION

Selectivity index of Parijoto extract showed higher in WiDr cell line. WiDr cell line is derivated of HT-29 human colon cell line, which is colon cancer p53 mutation cell[10]. While the HepG2 cell line was derivated from liver hepatocellular carcinoma of a 15-year-old Caucasian male[11], and the 4T1 cell line is a 6-thioguanine resistant cell line from mice[12].

Carcinogenesis of colorectal cancer arises from the combination of three mechanisms, such as chromosomal instability (CIN), CpG island methylator phenotype (CIMP), and microsatellite instability (MSI). As we know that in the classical mechanism of CIN, the pathway begins with APC (adenomatous polyposis coli) mutations which followed by activation of oncogene KRAS and inactivation of TP53, a tumor suppressor gene which is encoded of P53, a tumor suppressor protein[13]. The results of our study showed that biologically active compound of Parijoto extract gived a good activity in the proliferation of cancer cell lines, especially in WiDr.

Medinilla speciosa (the commont name of Parijoto) is one of Melastomaceae Family which is

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included in genus of Medinilla. The genus of Medinilla, specially *Medinilla magnifica* Lindley has been know to have polyphenol, specially ellagitannin, such as Medinillin A and Medinillin B. In another genus, ellagitannin has been found as ellagic acid, nobotanin D and the dimers nobotanins A,B and F, hydrolyzable tannins, casuarictin, pedunculagin, praeoxin A, B, casuarinin, and others[1]

Ellagitannin was hydrolyzed to become ellagic acid then it will be metabolized by colon bacteria to various urolithins. The ellagitannin and its derivates have been reported anti-cancer activities on HT-29 colon cell line[14], and the consumption of ellagitannin-containing pomegate extract (PE) was associated with the expression of CD44, CTNB1, CDKN1A, EGFR, and TYMs in a patient cancerous colon tissue[15].

Dietary polyphenol in colorectal cancer was given effect in apoptosis and chemoprevention[16]. Then Urolithin A showed can be induced celullar senescence p53-dependent manner in HCT-116 cells but not in other colon cancer cell lines with p-53 mutated or non-tumorigenic cell lines. That means that long-term senescence chemoprevention is p53-dependent manner[17].

## CONCLUSION

Ethanol extract of parijto fruits has been shown low and moderate activity in some cancer cell lines while it gave the greatest selectivity in WiDr cell line (colorectal cancer cell line) by in vitro study. Its means that parijoto extract is a promising cytotoxic agent for colorectal cancer therapy.

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