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RESEARCH ARTICLE

CYTOTOXIC EFFECT OF *Euphorbia tirucalli* EXTRACT TOWARDS BREAST MCF-7 AND COLORECTAL HT-29 CANCER CELL LINES

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ABSTRACT

Background: Cancer is a condition in which aberrant cells develop out of control. Breast cancer and colon cancer are two malignancies with significant occurrence rates. Radiation therapy, chemotherapy, hormone therapy, and surgery are a few of the therapeutic alternatives. Despite these obstacles, attempts have been made to investigate novel therapy methods, such as utilizing compounds found in plants. The pencil cactus (*Euphorbia tirucalli*) is a well-known medicinal plant with anti-inflammatory, antiviral, analgesic, and anticancer properties. According to earlier research, *Euphorbia tirucalli* has anticancer properties. ***Objective:*** The purpose of this study is to evaluate the cytotoxicity of Euphorbia tirucalli against MCF-7 breast cancer cells and HT-29 colorectal cancer cells. ***Methods:*** *Euphorbia tirucalli* was extracted using the maceration process using ethanol, n-hexane, and ethyl acetate as the solvents. Using the MTT test methods the cytotoxic activity of each extract against colorectal HT-29 and breast MCF-7 cancer cells was assessed. ***Results:*** Ethanol extract of *Euphorbia tirucalli* exhibited a moderate cytotoxic effects with an IC50 value of 33.88 g/mL toward breast MCF-7 cancer cells, and 34.86 g/mL toward colorectal HT-29 cancer cells, respectively. N-hexane extract of *Euphorbia tirucalli*had a low cytotoxic effect with IC50 values of 631.5 g/mL and 919.13 g/mL against breast MCF-7 and colorectal HT-29 cancer cells, respectively. ***Conclusion:*** It is important to continue developing *Euphorbia tirucalli* as a potential anti-breast and anti-colorectal cancer drugs as it exhibits cytotoxic effect against colorectal HT-29 and breast MCF-7 cancer cells.

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INTRODUCTION

Cancer is a type of disease with a high death rate which is characterized by a rapid growth of aberrant cells. In 2018, Cancer has caused 9.6 million death of the world's total population (Cancer *et al.,* 2018). It is estimated that cancer will affect about 19 million people in 2025 (New global cancer data, 2019). Based on World Health Organization (WHO) data in 2018, there are 348.809 cancer cases in Indonesia. Among women, breast cancer has the greatest incidence rate with 42.1 cases per 100,000 population, whereas colon cancer is included in the top ten highest incidence in Indonesia with 12.1 cases per 100.000 population (Indonesia cancer data, 2019). Treatments for cancer are radiation therapy, chemotherapy, hormone therapy, and surgery. However, these treatments have caused many side effects to human body such as anemia, edema, fatigue, nausea, constipation, and other body disorders (Side effect of cancer treatment, 2019). Therefore, alternative treatment is needed to reduce the mortality rate of cancer. The pencil cactus *(Euphorbia tirucalli)*, a species of medicinal plant, has been recognized for its many health advantages. *Euphorbia tirucalli* has been used in several countries for treating epilepsy, dyspepsia, jaundice, asthma, and tumor. *Euphorbia tirucalli* shows anti-inflammation, antiviral, analgesic, and anticancer properties, therefore, this plant becomes a prospecting candidate for new medicine (Gupta, 2023). Previous research has reported that *Euphorbia tirucalli* extract has shown anticancer activity against Mia-Pa-Ca2 pancreas cancer cells, KYSE 30 esophagus cancer cells. One of the compounds in *Euphorbia tirucalli*, euphol can inhibit antiproliferative and colony formation in pancreas cancer cell (Silva, 2018). This research will focus on the cytotoxic activity of *Euphorbia tirucalli* towardscolorectal HT-29 and breast MCF-7 cancer cells by MTT method.

MATERIAL AND METHODS

Materials: Pencil cactus, *Euphorbia tirucalli* (Pencil cactus, 2020) (Figure 1) were collected from Central Jakarta, Indonesia. HT-29 cancer cells are obtained from Cancer Chemoprevention Research Center, Yogyakarta, Indonesia. MCF-7 cancer cells are obtained from National Laboratory for Energy Conversion Technology, Serpong, Banten, Indonesia. Table 1 shows the taxonomy of *Euphorbia tirucalli* species (Mali, 2017).

Figure 1. Pencil Cactus (Euphorbia tirucalli)

Table 1. Taxonomy of *Euphorbia tirucalli*

Klasifikasi	Nama
Kingdom	*Plantae*
Division	*Tracheophyta*
Classis	*Manoliopsida*
Ordo	*Malphighiales*
Familia	*Euphorbiaceae*
Genus	*Euphorbia*
Species	*Euphorbia tirucalli*

METHODS

Cytotoxic Activity Evaluation by MTT assay: In this research, cytotoxic effect of *Euphorbia tirucalli*extracts against colorectal HT-29 and breast MCF-7 cancer cells is assessed using the MTT method. MTT assay is a colorimetric assay that relies on the reduction of yellow tetrazolium MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide into the purple crystals of formazan. The NADP (H) dependent enzyme that can reduce the yellow tetrazolium MTT into the purple crystal of formazan is produced by metabolically active cells (MTT, 2019). At the beginning, the cancer cells will be cultured in DMEM. Then the cultured cells are added to plate containing 96 micro-well. Each wellis containing 10^4 cells. The plate will be incubated for 24 hours. Then, 100 µg /ml PBS will be added to remove the medium and wash the cells (Meiyanto, 2019). In this present research, three extracts of Euphorbia tirucalli, namely ethanol extract, n-hexane extract, and ethyl acetate extracare used. *Euphorbia tirucalli* extracts were diluted until reach the concentrations of 1.56, 3.125, 6.25, 12.5, 25, 50, 100, 200 µg /mL cells. These extract samples were added to the target ofMCF-7 cells. The experiment to evaluate cytotoxic activity to the breast MCF-7 cancer cells was done three times (triplo). *Euphorbia tirucalli* were also diluted until reach the concentrations of 3.90625, 7.8125, 15.625, 31.25, 62.5, 125, 250, 500µg /mL and these various concentrations were added to the target of HT-29 cells. The experiment to evaluate cytotoxicity of *Euphorbia tirucalli* extracts towards to HT-29 cells was done twice (duplo). Following a 24-hour incubation period, the cells were mixed with 100 L of 5 mg MTT reagent in a 10 ml phosphate buffered saline solution. The plate was then left incubating for 4 hours. After that, the cells were examined with microscope, if the purple crystals of formazan were developed, in amount of 100 µLDMSO (dimethyl sulfoxide) was introduced to dissolve the sediments. The absorbance is read using 630 nm on the spectrophotometer. After that, the percentage inhibition is calculated by the formula (Meiyanto, 2019 and Arsianti, 2016):

$$\%\ \text{Inhibitica} \frac{(\text{absorbance of negative control-absorbance of treatment})}{\text{Absorbance of negative control}} x$$

IC_{50} value is the concentration of extract which inhibits 50% of cancer cell growth. The IC_{50} value is calculated using the linear regression equation; y = ax+b between the percentage of inhibition on cancer cell line in y axis and log concentration of the extract in x axis, by substituting the a and b value from the linear equation to the formula of $IC_{50} = 10^{(50-b)/a}$ (12).

RESULTS

Cytotoxic Activity of Euphorbia tirucalli extracts Towards Breast MCF-7 Cancer Cells: Figure 2 shows the linear correlation between concentration of ethanol extract of*Euphorbia tirucalli* with the percentage of inhibition on breast MCF-7cells for trial 1, trial 2 and trial 3 (triplo). The IC_{50} value is calculated by using linear equation of ethanol extract of *Euphorbia tirucalli* based on triplo experiment that have been described previously. The equations arey = 12.607x +27.447 (trial 1); y = 5.4811x + 41.468 (trial 2), and y = 19.399x + 38.084 (trial 3). The IC_{50}values of ethanol extract which calculated by using the IC_{50} formula are 61.51µg/mLfor trial 1, 36.03µg/mLfor trial 2, and 4.11µg/mLfor trial 3, respectively, with the average of IC_{50} value is 33.88 µg/mL. Figure 3 displays the linear correlation between concentration of n-hexane extract of *Euphorbia tirucalli* with percentage of inhibition on breast MCF-7 cancer cells for trial 1, trial 2, and trial 3.The linear equations are y = 12.756x +11.792 (trial 1); y = 20.348x + 12.289 (trial 2); y = 11.364x + 16.801 (trial 3). The IC_{50} values of n-hexane extract which calculated by using the IC_{50} formula are 989.23µg/mL for trial 1, 70.80 µg/mL for trial 2, and 843.48 µg/mLfor trial 3 respectively, with the average of IC_{50} value is 631.5 µg/mL. Figure 4 shows the correlation between concentration of ethylacetate extract of *Euphorbia tirucalli*with percentage of inhibition onbreast MCF-7 cancer cells for trial 1, trial 2 and trial 3. The linear equations are y = 5.5417x +33.186 (trial 1);y = 6.2918x + 27.327 (trial 2); y = 3.9287x + 36.305 (trial 3). The IC_{50} values of ethylacetate extract which calculated by using the IC_{50} formula are 1081.65 µg/mL for trial 1, 4014.02 µg/mLfor trial 2, and 2745.5µg/mLfor trial 3, respectively, with the average of IC_{50} value is 2613.7µg/mL.

Cytotoxic Activity of Euphorbia tirucalli extracts against Colon HT-29 Cancer Cells: Figure 5 shows the correlation of concentration of ethanol extract of *Euphorbia tirucalli* with percentage of inhibition against HT-29cells. IC_{50} value is calculated by using linear equation of ethanol extract of *Euphorbia tirucalli* based on duplo experiment (trial 1 and trial 2) that have been mentioned previously. The equations of ethanol extract of*Euphorbia tirucalli* are y = 35.02x – 0.6643 (trial 1) andy = 66.368x -57.557 (trial 2). The IC_{50} values of the ethanol extract which calculated by using the IC_{50} formula are 27.97µg/mL (trial 1) and 41.75 µg/mL (trial 2), respectively, with the average of the IC_{50} value is 34.86 µg/mL. Figure 6 shows the linear correlation of the concentration of n-hexane extract of*Euphorbia tirucalli* with percentage of inhibition against colorectal HT-29 cancer cells for trial 1 and trial 2. The equations are y = 17.763x – 7.9857 (trial 1) and y = 137.62x -73.316 (trial 2). The IC_{50} values of n-hexane extract which calculated by using the IC_{50} formula are 1838.27µg/mL (trial 1) and 7.87 µg/mL (trial 2), with theaverage of IC_{50} value is 919.13µg/mL. Figure 7 shows the correlation of concentration of ethylacetate extract of *Euphorbia tirucalli* with percentage of inhibition against colorectal HT-29 cancer cells for trial 1 and trial 2. The linear equations are y = 17.763x – 7.9857 (trial 1) and y = 137.62x -73.316 (trial 2). The IC_{50} values of ethylacetate extract which calculated by using the IC_{50} formula are 5824.06µg/mL(trial 1) and 7956.54 µg/mL (trial 2), respectively, with the average of IC_{50} value is 6890.3µg/mL.

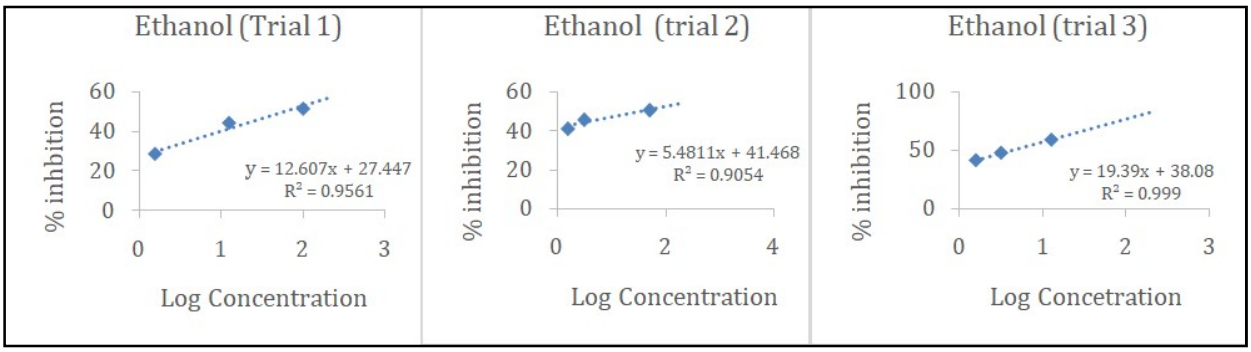

Figure 2. Linear equation of ethanol extract of *Euphorbia tirucalli* (trial 1-3) on breast MCF-7 cells

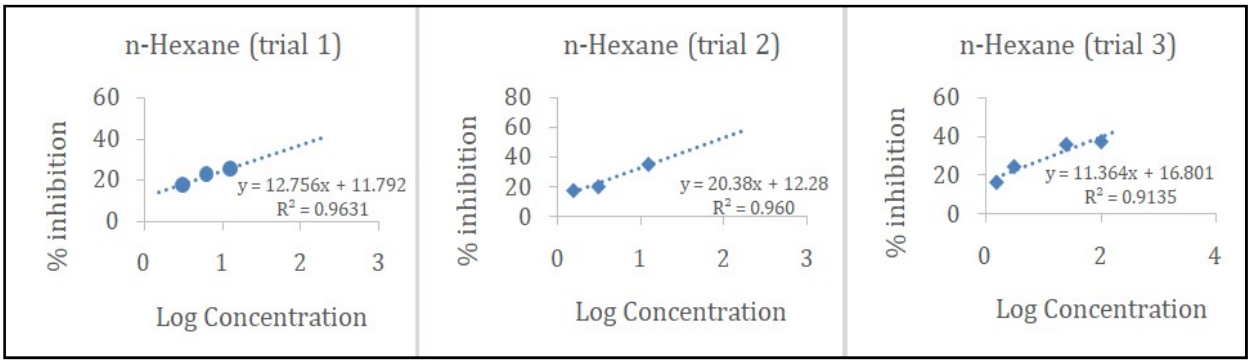

Figure 3. Linear equation of n-hexane extract of *Euphorbia tirucalli* (trial 1-3) on MCF-7 cells.

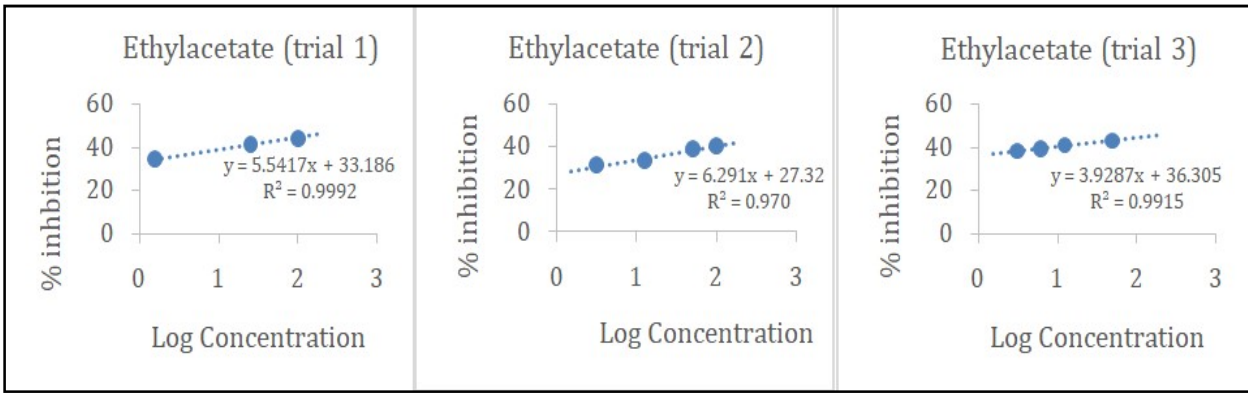

Figure 4. Linear equation of ethyl acetate extract of *Euphorbia tirucalli*(trial 1-3) on MCF-7 cells

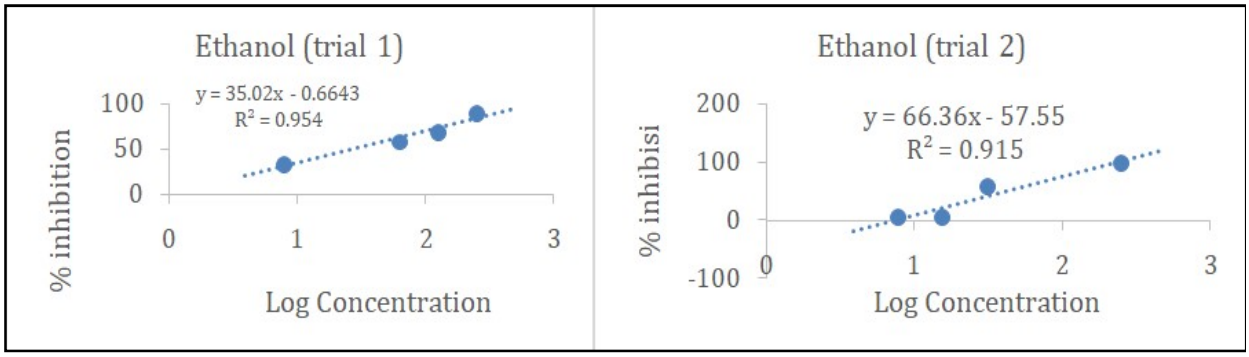

Figure 5. Linear equation of ethanol extract of *Euphorbia tirucalli* (trial 1-2) against HT-29 cells

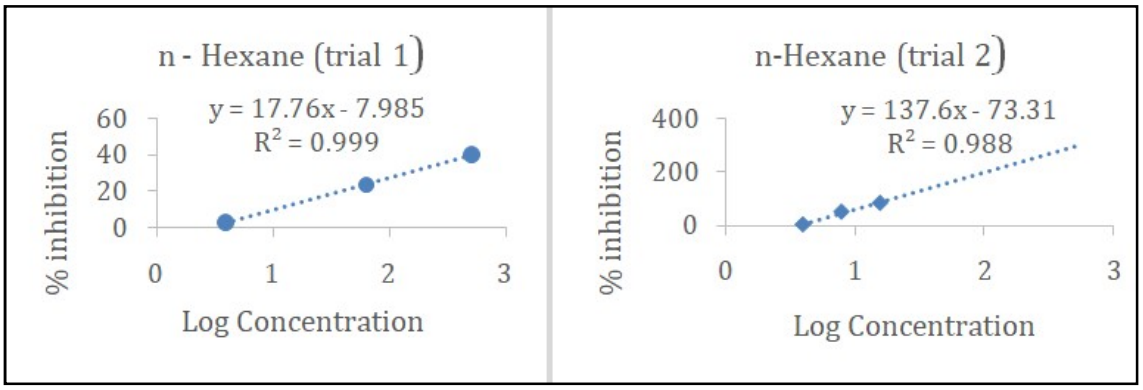

Figure 6. Linear equation of n-hexane extract of *Euphorbia tirucalli* (trial 1-2) against HT-29 cells

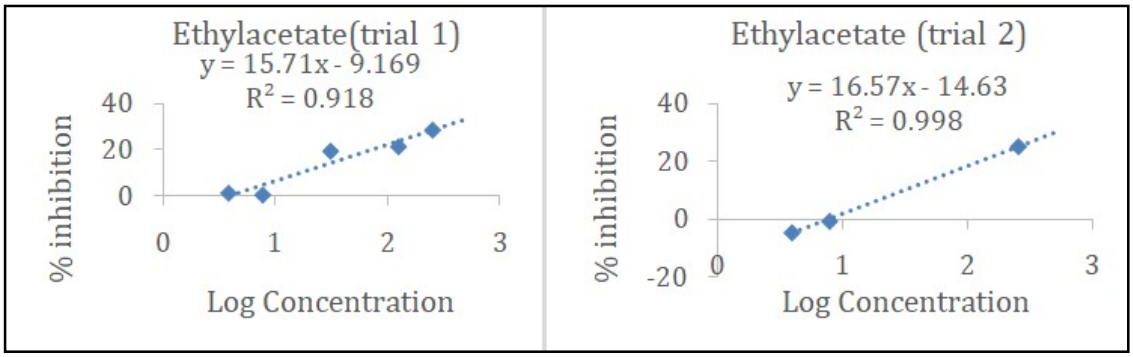

Figure 7. Linear equation of ethylacetate extract of *Euphorbia tirucalli*trial 1-2) on HT-29 cells

Table 2. Cytotoxic effect of *Euphorbia tirucalli* extracts and doxorubicin against MCF-7 cells

Extract of *Euphorbia tirucalli*	IC_{50} (µg/mL) on HT-29 cells
Ethyl Acetate	6890.30
n-Hexane	919.13
Ethanol	34.86
Doxorubicin (positive control)	488.61

Table 3. Cytotoxic effect of *Euphorbia tirucalli* extracts and doxorubicin against HT-29 cells

Extract of *Euphorbia tirucalli*	IC_{50} (µg/mL) on HT-29 cells
Ethyl Acetate	6890.30
n-Hexane	919.13
Ethanol	34.86
Doxorubicin (positive control)	488.61

DISCUSSIONS

Figure 2-4 show the correlation of concentration of ethanol extract, ethylacetate extract and n-hexane extract of *Euphorbia tirucalli*, which is directly proportional to its percentage of inhibition against breast MCF-7 cells. Based on linear regression on Figure 2-4, we conclude that cytotoxic activity of *Euphorbia tirucalli* extracts towards MCF-7 cells follow dose-dependent manner, which means the greater concentration of extract, thegreater percentage of inhibition. Table 2 summarize the cytotoxic activity of *Euphorbia tirucalli* extracts toward MCF-7 cells, showed in IC_{50} value. The IC_{50} or half maximal inhibitory is defined asthe concentration of the extract that may inhibit 50% of the development of cancer cells.Based on study by Atjanasuppat *et al*, the IC_{50} value can be classified into 4 classes, as follows : IC_{50} value of the extract over 1000 µg/mL is classified to have no cytotoxic effect, the extract is considered to have a weak cytotoxic effect if its IC50 value is between 100 and 1000 g/mL; if it is between 20 and 100 g/mL, it is considered to have a strong cytotoxic effect (Atjanasuppat, 2009). As shown in Table 2, with an IC50 value of 33.88 g/mL, the ethanol extract of *Euphorbia tirucalli* exhibits a moderate cytotoxic effect on breast MCF-7 cells.

Doxorubicin as control positive and n-hexane extract of *Euphorbia tirucalli* with IC_{50} of 191.63 µg/mL and 631.50 µg/mL respectively, are assigned to have a weak cytotoxic effect. Ethylacetate extract of*Euphorbia tirucalli* with IC_{50} value of 2613.72 µg/mL demonstrated no cytotoxicity effect against breast MCF-7 cancer cells. Based on thsese results, ethanol extract is more effective as an anti-breast cancer on MCF-7 cells compared to doxorubicin, n-hexane extract, and ethylacetate extract of *Euphorbia tirucalli*. According to previous researchby Choene et al., it has been reported that *Euphorbia tirucalli* demonstrate growth inhibitory and cancer cell killing effect on cancer cells of breast MCF-7 and breast MDA-MB 231. The extracts triggered apoptosis in cell lines and blocked the cell cycle at the G0/G1 phase, also upregulated the expression of p21 in both cells which speculate the extracts reduced cell growth by arrest the cell cycle due to upregulation of p21 that can suppress cyclins (Choene, 2016). Furthermore, a study by Silva VAO, et al reported that euphol, an active compound which has been purified from *Euphorbia tirucalli* extract, demonstrated an anticancer effect on MCF-7 cancer cells with IC_{50} value of 18.76 µg/mL (Silva, 2018). Figure 6-8 show the linear relationship between the concentration of ethanol, ethylacetate,and n-hexane extracts of *Euphorbia tirucalli*with its percentage of inhibition

againstcolorectal HT-29 cancer cells. Based on this linear relationship, we conclude that cytotoxic activity of *Euphorbia tirucalli* towardscolorectal HT-29 cancer cells followed the dose-dependent manner, which is the greater concentration of the extract, the higherpercentage of inhibition. Cytotoxic effect expressed by IC_{50} value of *Euphorbia tirucalli*extracts and doxorubicin against colorectal HT-29 cancer cells is summarized in Table 3. As shown in Table 3, ethanol extract of *Euphorbia tirucalli*with IC_{50} value of 34.86 µg/mL exhibits a moderate cytotoxicity on colorectal HT-29 cells. Doxorubicin as a positive control with IC_{50} value of 488.61 µg/mL, has a weak cytotoxic effect on HT-29 cells. Similar with doxorubicin, n-hexane extract of *Euphorbia tirucalli*with IC_{50}value of 919.13 µg/mL, also hasa weak cytotoxic effect on HT-29 cells. Ethylacetate extract of*Euphorbia tirucalli*did not show cytotoxic effect towards colorectal HT-29cells since its IC50 value of 6890.30 µg/mL. Based on the results, ethanol extract of *Euphorbia tirucalli*is more effective as an anti-colorectal cancer against HT-29 cells compared to doxorubicin, n-hexane extract and ethylacetate extract of *Euphorbia tirucalli*. Previous research by Silva VAO, et alreportedthat ingenol B,isolated compound from *Euphorbia tirucalli*hadanticancer activity towards colorectal HT-29 cells with IC_{50}value of 41.83 µg/mL (16).Furthermore, another studyaccomplished by Silva VAO, et al reported that euphol,an active compound that has been purified from *Euphorbia tirucalli* extract had anticancer activity on HT-29 cancer cell with IC_{50} value of 6.50 µg/mL (Silva,). Another study by Achanjo AB, et al reported that there was an increasing in expression of caspase 3 and p-53 in another colorectal cancer cell line, namely HCT-116, which was exposed to *Euphorbia tirucalli* extracts. Expression of protein p-53 indicate the cells are undergoing apoptosis and the activation cascade of caspase including caspase 3 is known as hallmark event of apoptosis (Archanjo, 2016).

CONCLUSION

Ethanol extract of pencil cactus (*Euphorbia tirucalli*) ethanol extract, which had a moderate cytotoxic effects against the breast cancer cell line MCF-7 and the colorectal cancer cell line HT-29, can potentially be further developed into powerful anti-breast and anti-colorectal cancer drugs.

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