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Research Article

CYTOTOXIC ACTIVITY OF *ERIOCAULON CINEREUM* R.BR TO MCF-7 AND VERO CELL LINE

ARDE TOGA NUGRAHA*, ASGAR PURNAMA, SITI NURUL KOMARIAH, HADY ANSHORI T.

Department of Pharmacy, Universitas Islam Indonesia, Yogyakarta, Indonesia. Email: arde.toga@uii.ac.id

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ABSTRACT

Objective: The aim of the study was to determine the strength of the *Eriocaulon cinereum* R.Br plant against breast cancer cells (MCF7) and cytotoxic against Vero cells.

Methods: *E. cinereum* R.Br was taken from the province of Bangka Belitung and then extracted in stages with n-hexane, ethyl acetate, and methanol. Then, the ethyl acetate extract was fractionated using the vacuum liquid chromatography method with dichloromethane and ethyl acetate solvents. Sample was tested for MCF-7 cells and Vero cells using the MTT method (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide). The data obtained is analyzed by probit SPSS.

Results: The results of this study showed that the best IC_{50} extract was ethyl acetate extract with an IC_{50} value of 450.31 µg/ml. Then, ethyl acetate extract produced dichloromethane fraction with IC_{50} value of 443.52 µg/ml and ethyl acetate with IC_{50} value of 214.75 µg/ml. Ethyl acetate fraction was also tested against Vero cells to see cytotoxic against normal human cells with IC_{50} 679.11 µg/ml

Keywords: *Eriocaulon cinereum* R.Br, MCF-7, Vero, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.

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INTRODUCTION

Cancer is an autonomous tissue growth that does not follow the rules and regulation of normal cells, where there is a disruption or failure of the multiplication regulation mechanism in multicellular organisms resulting changes in uncontrolled cell behavior [1]. Breast cancer is the most common cancer in women and induced by environmental, genetic, and epigenetic risk factors [2]. Worldwide, breast cancer covers 25.5% of all cancers in women [3]. According to the National Cancer Institute, in 2011, more than 200,000 women were diagnosed with breast cancer in the United States [2]. In 2013, the prevalence of breast cancer was 1.4% or an estimated 347,792 people in the population of all ages in Indonesia [4].

Breast surgery and mastectomy are the definitive treatment for this disease. Treatments such as radiation therapy, chemotherapy, and hormonal therapy are prescribed to reduce disease recurrence and metastasis [2]. Therefore, effective alternative therapies for cancer are needed with minimal side effects. Plant metabolite compounds have shown characteristic inhibition of cancer cell activity, such as inhibiting the proliferation of cancer cells and inducing apoptosis [5].

The Bangka-Belitung people in Indonesia was used *Eriocaulon cinereum* R.Br (which is traditionally known as grass gong) as an anticancer drug. Grass or *E. cinereum* R.Br is one of 400 species of *Eriocaulon* form of herbaceous plants such as grass with wet or watery soil habitats. This plant has been also empirically long used by Chinese people as a companion therapy in cancer treatment. The *Eriocaulon* genus was often used to prevent abnormal cell growth. This plant was also applied as a companion compound in tumor therapy by Chinese people from generation to generation [6]. Other research on the genus eriocaulon also discusses phenolic and steroid content which might be responsible as a cytotoxic agent [7,8].

In this study, a cytotoxic activity of dichloromethane and ethyl acetate fraction from ethyl acetate extract of grass gong (*E. cinereum* R.Br) on MCF-7 and Vero cells was carried out. The aim of study in the form of fractions was separated the main group of ingredients from one of the other main groups of content which is expected to have cytotoxic activity that is better than the extract form. MCF-7 cell is a breast cancer cell model that widely used in research and use of Vero cells in this study aimed as a comparison to determine the selectivity of the fraction of MCF-7 cells. Vero cells are also easy and fast to replicate and do not require strict culture conditions to support their proliferation [9].

MATERIALS AND METHODS

Materials

This plant was collected from Parittiga, Jebus, Bangka Belitung Island Province, Indonesia, on July 2016. *E. cinereum* was identified in the plant systematic laboratory, Faculty of Biology, Gadjah Mada University, Indonesia. Samples have been collected then go through the sorting process and dried at a 50°C. Then the dried plant was mashed to powder.

Instrumentation

The tools used in this study were glassware, refrigerators, (Laminar Air Flow, Labconco), micropipettes, analytical scales (Mettler Toledo), vortex, a set of vacuum liquid chromatography (VLC) devices, inverted microscopes, hemasitometers (Neubauer), tube, dispersing machines, CO_2 incubators (HERA cell) and liquid nitrogen frames, ELISA readers, cabinet dryers, rotary evaporators (Heidolph-L4000), desiccators, fume hoods, sonicator devices, ovens, UV254, and UV366 lamps.

Preparation of plant extract

The extraction process was done using modified ultrasound-assisted extraction [10]. 37.5 g of raw *E. cinereum* R.Br was put into a 500 **ml** beaker and added 375 **ml** of n-hexane. The *E. cinereum* R.Br has been added to the solvent and then put into a sonicator device that has been set temperature at 40°C and carried out for 30 min.

The extract obtained was separated from the pulp with filter paper and Buchner funnel. The pulp was re-extracted with 500 mL ethyl acetate using the ultrasound-assisted extraction method with a sonification device at 40°C for 30 **min**. The extract obtained is separated from the pulp with filter paper and a Buchner funnel. Extraction was continued using 50 **mL** of methanol as well as the ultrasound-assisted extraction method to produce the most polar extract. N-hexane, ethyl acetate, and methanol extract were then thickened and dried using a rotary evaporator.

Fractionation of ethyl acetate extract

The ethyl acetate extract of gong grass was fractionated using VLC with a stationary phase in the form of GF254 silica and mobile phase comparison that had been modified from the study [6]. The solvent used was 100 ml dichloromethane and then 100 ml ethyl acetate.

Cytotoxic activity

The *E. cinereum* R.Br was evaluated with MCF7 and Vero cell line to determine cytotoxic activity [11]. All extracts (n-hexane, ethyl acetate, and methanol) were evaluated first to get the best activity and then were done fraction of the extract for the best cytotoxic activity against MCF7 cells. The fraction that had the best activity was also evaluated for Vero cells. The sample was dissolved in DMSO at a concentration of 500 µg/ml, 250 µg/ml, 125 µg/ml, 62.5 µg/ml, 31.25 µg/ml, 15.62 µg/ml, 7.81 µg/ml, and 3.90 µg/ml. Growth media (Roswell Park Memorial Institute 1640) suspension with cells was centrifuged at 1500 rpm for 10 min. The supernatant was poured off and resuspended in 10 ml media and incubated in the CO_2 incubator at 37°C. Cells were observed under the microscope and harvested if the number of cells enough (confluent). 100 µl cells were seeded at 10,000–50,000 cells/ml placed in 96-well plates, 24 h later, added the 100 µl sample. Then, the plate was incubated for 24 h in CO_2 incubator. The media were removed from the well, and 100 µL of a new medium and 10 µL MTT (0.5%) were added. The plate was incubated for 3 h and then added 10% SDS. The plate was incubated 4 h and kept in dark place at room temperature. The absorbance was measured by Elisa reader at wavelength 550 nm.

Determine of phytochemical compounds from active fraction

Determine chemical constituents of the fraction were used a reagent spray Dragendorff Anisaldehyde-Sulfuric acid, $FeCl_3$, $AlCl_3$, and Liebermann–Burchard test [12].

RESULTS AND DISCUSSIONS

Evaluation of cytotoxic activity against MCF-7 cells was carried out with MTT assay which can be used to measure colorimetric cell proliferation. The reaction of MTT assay is that formazan violet formed from the reduction reaction of MTT compounds with mitochondrial reductase enzymes in living cells. The more cells that live, the higher the absorbance value, whereas if the absorbance value is small, it indicates that there is cell death. Absorbance readings were performed using ELISA reader at a wavelength 595 nm.

The results of n-hexane, ethyl acetate, and methanol extract on MCF7 cells obtained the best IC_{50} value in ethyl acetate extract of 450.31 µg/ml (Table 1). Ethyl acetate extract produced from 37.5 g of raw material of *E. cinereum* R.Br is 2.52 g (6.73%). Furthermore, the extract with the best activity against MCF7 cells was fractionated using the VLC method. The mobile phase used in the fractionation process is dichloromethane and ethyl acetate. Fractionation begins with using dichloromethane (100 ml) and then proceeds with ethyl acetate (100 ml). The two fractions were then tested against MCF7 cells and Vero cells.

The results of both dichloromethane and ethyl acetate fractions showed a fairly good value, and dichloromethane fraction has IC_{50} value of 443.52 µg/ml. While ethyl acetate fraction only has IC_{50} value of 214.75 µg/ml, the results of ethyl acetate fraction against Vero cells also showed good results with IC_{50} value of 679.11 µg/ml. This means ethyl acetate fraction has good activity against MCF7 cells but also has less toxic effect on normal human cells (Vero cells). The other species of *Eriocaulon* as *Eriocaulon sieboldianum* was used as adjuvant cancer therapy [6]. Previously, other species of *Eriocaulon* had also been reported to have cytotoxic effects on MCF-7 cells. One of them is *Eriocaulon australe* from China, this extract was reported to has a cytotoxic effect on A549, MCF-7, and HeLa Cells [13].

Table 1: Result of cytotoxic evaluation

Sample	IC_{50} against MCF7 cells	IC_{50} against Vero cells
n-hexane extract	>1000 µg/ml	n/a
Ethyl acetate extract	450.31 µg/ml	n/a
Methanol extract	>1000 µg/ml	n/a
Dichloromethane fraction	443.52 µg/ml	413,04 µg/ml
Ethyl acetate fraction	214.75 µg/ml	679.11 µg/ml

*n/a=not available

IC_{50} value is the concentration value which results in 50% cell proliferation resistance and shows the potential for toxicity of a compound. The greater the IC_{50} value the lower the potential of a compound as a cytotoxic agent: IC_{50} value ≤20 µg/ml=very active, IC_{50} 21–200 µg/ml=quite active, IC_{50} 201–500 µg/ml=weak active, and IC_{50} >501 µg/ml=inactive [14]. IC_{50} cytotoxic evaluation results were obtained based on probit test using SPSS; the data obtained was made into the equation of the relationship between concentration as X and percent cell death as Y.

The fraction with the best activity was identified the phytochemical compound. Identification was carried out using a thin layer of chromatography and then sprayed with reagent Dragendorff, anisaldehyde-sulfuric acid, FeCl3, AlCl3, and Liebermann-Burchard. The stationary phase was used silica gel 60 GF254, and the mobile phase was used n-hexane:ethyl acetate (8:2).

The results of the observation show that ethyl acetate fractions had positive contain phenolic, steroids, and terpenoids. This was shown by the appearance of a green color which indicates a positive containing phenolic compound at (Rf 0.75) with $FeCl_3$ spray reagent. Based on previous research, ellagic acid as phenolic compounds which were tested on colon cancer cells was able to inhibit cancer cell growth by inhibiting apoptosis and proliferation of cancer cells [15]. In addition to the phytochemical testing using Liebermann–Burchard reagent for steroid detection, ethyl acetate fraction showed positive results with green color (Rf 0.46) after heating at 100°C for 5 min. In a previous study, the content of exemestane compounds included as a steroid group was shown to be able to inhibit the growth of cancer cells by inducing apoptosis and inhibiting proliferation, which has been tested *in vitro* on breast cancer cells with IC_{50} values of 42 µg/ml [16]. The other phytochemical content of ethyl acetate fraction using anisaldehyde-sulfuric acid reagent looks positive terpenoid compound at the value of Rf 0.42 with the resulting purple color. From several previous studies, it has been proven that pseudolaric B acid as terpenoid compound has been shown to be able to inhibit cancer cell growth by inhibiting the penetration of tested microtubules in breast cancer cells (T47D) with an IC_{50} value of 1 µg/ml [17]. Negative results on phytochemical testing were obtained by testing alkaloid compounds with Dragendorff reagents and flavonoids with $AlCl_3$ reagent.

The results that have been carried out show the potential of the *E. cinereum* R.Br plant as one of the additional alternatives for cancer treatment. Ensuring potential can be done further by finding the main compounds in the plant that has cytotoxic effects. The search for the main compound can be done by isolation using preparative high-performance liquid chromatography and identification with nuclear magnetic resonance. So that, in the future, it can be traced from ethyl acetate fractions to find which compounds are responsible and can be synthesized to help treat cancer. In addition, from the results of this study, these plants can also be used traditionally for additional therapy to fight cancer cells.

CONCLUSIONS

The results of cytotoxic against MCF7 cells from ethyl acetate fraction showed IC_{50} values 214.75 µg/ml. This shows good potential for the sustainability of research in finding active compounds that provide cytotoxic effects. In addition, it also has the potential to be one of the additional therapies against cancer in the future.

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