Cytotoxic activity of flavonoid from local plant *Eriocaulon cinereum* R.B against MCF-7 breast cancer cells

**Abstract**

*Eriocaulon cinereum* R. Br is used as traditional medicine by the local community in Bangka Belitung Island, Indonesia. The plant is processed as an infusion for fever, boosts the immune system, and treats tumor cells. However, scientific research on this species is still limited. The aims of this study were to determine the cytotoxic of *E. cinereum* against MCF-7 cells. The results suggested that one of the compounds has a good cytotoxic activity. Therefore, it is quite promising in the effort of cancer drug discovery. The active compound has a flavonoid, which plays a role in several anticancer mechanisms. This study provided scientific evidence regarding the utilization of *E. cinereum* by the local community for cancer therapy. The plant can be further developed as an alternative agent to treat cancer or as cancer adjuvant therapy.

**Key words:** Cytotoxic, *Eriocaulon cinereum* R. Br, Flavonoid, MCF-7

**INTRODUCTION**

*Eriocaulon cinereum* R. Br belongs to the Eriocaulaceae family. This plant has fronds measuring 2–4 cm and 15–30 cm in height, tapered tips, and thin straight leaves up to 8–10 cm long.[1] *E. cinereum* extract and fractions are known to have activity against breast and cervical cancer cells. Based on the literature, this plant can be developed as an anticancer agent with minimal toxicity.[2-4] Information about *E. cinereum* is still minimal compared to other species of the genus. With many similarities to *E. cinereum*, *Eriocaulon sieboldianum* was used as supportive therapy for cancer treatment in China. This plant is processed together with other herbal plants to treat diseases, such as to reduce fever, protect the eyes, and reduce fat levels in the blood.[5,6] *E. sieboldianum* extract is known to inhibit cell proliferation and apoptosis induction. This plant has the potential as a dietary supplement with antitumor activity. It is also a target for developing anticancer compounds with low toxicity.[7]

Breast cancer is a condition where cells cannot recognize themselves normally; abnormal cells occur in supporting tissues, glandular cells, and glandular channels.[8] Breast cancer is the most common cancer in women. There are eight main features associated with this disease, including continuous proliferation, avoidance of apoptosis, increased invasion, increased metastasis, initiation of inflammation,
induction of angiogenesis, metabolic disorders, and immune dysregulation. Breast cancer is the most common disease in the female population, which is 24.2% of the cancer patients with breast cancer in the world. Furthermore in Indonesia, breast cancer is the highest rank cancer with a total of 43.2% of women with all cancer patients, with a mortality rate of 12.9%. The death rate is quite high due to discomfort during therapy and the habits of Indonesian people who prefer traditional medicine without proper scientific evidence. This could be due to breast cancer therapy resistance, and additional treatment may increase its effectiveness.

MCF-7 cells are among the most common cells used to test and study breast tumors and cancers in vitro. These cells were taken from the breast tissue of a Caucasian woman with blood Type O with positive rhesus. MCF-7 cells can be grown in Dulbecco’s modified Eagle medium (DMEM). In MCF-7 cell culture, estrogen is processed in the form of estradiol through estrogen receptors in the cell cytoplasm. Despite various limitations, MCF-7 cells have been reliable in vitro tests for more than 40 years. These cells are also essential in using experimental animals in breast cancer trials. However, it is necessary to consider various conditions for the validity of results. In contrast, Vero cells were taken from mammals and are often used in molecular biology research. These cells are noncancerous epithelial cells originating from the kidney of African green monkeys, which are flat monolayer cells. Vero cells are also often used in virological research for parasites (Neospora), chemical effect tests, and toxin studies of intracellular bacteria.

The aims of this study were to find compounds that are responsible for cytotoxic activity against MCF-7 cells from E. cinereum, and also prove their empirical use in the community.

MATERIALS AND METHODS

Plant collection and sample preparation

E. cinereum R. Br was grow at Parittiga, Jebus, Bangka Belitung Island, also collected in January 2018, and identified by Dr. Heri Sujadmiko (Laboratory of Plant Systematics, Faculty of Biology, Gadjah Mada University, Yogyakarta, Indonesia). The voucher specimen (BF-FAUII-01) was deposited at the Laboratory of Pharmaceutical Biology, Department of Pharmacy, Universitas Islam Indonesia (Yogyakarta, Indonesia).

After sorting, the plants were chopped and dried in a drying cabinet at 50°C for 3 days. The extraction was done using the modified ultrasound-assisted extraction method. The extraction and fractionation method was carried out following previous studies. E. cinereum (1 kg) was extracted with n-hexane three times (30 min each).

Then, the pulps were extracted again with ethyl acetate three times (30 min each). Both extracts were concentrated using a vacuum rotary evaporator to obtain the crude extract of n-hexane (5 g) and ethyl acetate (50.4 g).

The potential crude extract (based on previous research), ethyl acetate extract, was separated by chromatography vacuum column. The fractionation used dichloromethane and ethyl acetate as mobile phases. The fractionation of 35 g ethyl acetate extract produced dichloromethane fraction (13.6 g) and ethyl acetate fraction (7.5 g). Then, the potential fraction (based on previous research), dichloromethane fraction, was separated by preparative thin-layer chromatography (TLC) with n-hexane: ethyl acetate (8:2) as the mobile phase. The separation resulted in three dominant bands (SF1–SF3).

SF1 and SF2 were isolated using semi-preparative reversed-phase high-performance liquid chromatography (RP-HPLC). All peaks that appear on the chromatogram are taken and tested for compound content. So that obtained isolates of flavonoids and terpene. Both of the compounds were tested on MCF-7 cells.

Cell lines

MCF-7 and Vero cell lines were cultured at the Parasitology Laboratory, Medical Faculty, Gadjah Mada University. MCF-7 cells were cultured in DMEM. Vero cells were cultured in M199 medium. The cells were maintained at a CO₂ incubator at 37°C.

Cytotoxic activity evaluation

The cytotoxic activities of the subfractions (SF1–SF3) and isolate (flavonoid and terpene) on MCF-7 were evaluated using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. These subfractions were dissolved in dimethyl sulfoxide to a final concentration in 500 μg/mL and isolate in 50 μg/mL to produce serial concentrations for screening. About 50 mg MTT powder was dissolved in phosphate-buffered saline (PBS; 10 mL). The MTT reagent (0.5 mg/mL) was prepared for treatment by taking 1 mL MTT stock in 5 mg/mL PBS. The reagent was diluted with complete DMEM (10 mL). The cell medium on the plate was removed, and then washed with PBS. The prepared MTT reagent (100 μL) was added to each well plate, including the control medium, and then incubated until formazan salt crystals formed in a 5% CO₂ incubator for 4 h. The condition of the cells was observed using an inverted microscope. If formazan salt crystals were formed, 10% sodium dodecyl sulfate stopper reagent was added to 0.01 N Hydrochloric acid (HCl) (100 μL). The plates were then wrapped in aluminum foil and incubated in the dark at room temperature for ±12 h. After incubation, absorbance was measured using an enzyme-linked immunosorbent assay reader. Cell viability was done as follows: Cell viability (%) = (OD (compounds) − OD [control]/OD [control]) ×100%. All assays were performed at least in triplicate.
Statistical analysis
The data obtained from the cytotoxic activity evaluation were analyzed with PROBIT from the IBM SPSS Software ver. 19.0 IBM Corp., USA).

RESULTS

Cytotoxic activity
Table 1 presents the cytotoxic activity data of the subfraction and isolate. These results showed that flavonoid has the best activity value, with an IC₅₀ of 7.28 ± 6.75 μg/mL in MCF-7 cells. The power of this toxicity is quite good in describing how these compounds can kill cancer cells. Of course, this is the best result when compared to other samples, also in line with the positive control.

Phytochemical screening
The identification of the active compound (flavonoid) was also performed using reverse-phase HPLC (Waters®, stationary phase C18 (Xterra®) with a PDA detector. In Figure 1. The flavonoids were isolated from their peak at a retention time of 32,793 [Figure 1a] and then their purity was identified by RP-HPLC, the purity level was known to be 75.58% [Figure 1b]. The maximum wavelength of flavonoids obtained from the photodiode-array detection detector is 273.5 nm and 376 nm [Figure 1c]. This data was also supported by qualitative identification from the spray reagent with AlCl₃ [Figure 1].

DISCUSSION

Traditional medicine has long been used to treat many diseases in Indonesia. Traditional medicine demonstrated many obvious advantages of anticancer therapy using multiple target mechanisms and types. One of the widely used plants as traditional medicine has already completed clinical trials and is recommended for cancer prevention and therapy. E. cinereum is traditionally used by the Bangka Belitung Island community for cancer treatment. The development of traditional medicine from this plant is still in the early stage. In this study, the subfraction of E. cinereum R. Br demonstrated cytotoxic activity against MCF-7. Subfractions 1 and 2, then isolated produce flavonoid and terpene compounds. Both of the compounds were also evaluated to MCF-7.

As shown in Table 1, E. cinereum R. Br SF1 and SF2 exhibited cytotoxic activity against MCF-7 cancer cells. Interestingly, the SF2 has a higher cytotoxic effect than SF1 on MCF-7. However, the final result of best cytotoxic activity precisely flavonoid, isolated from SF1. As shown in Table 1, flavonoid has IC₅₀ 7.28 ± 6.75 μg/mL. These results show that against 50% of MCF-7 cancer cells only 7.28 μg/mL is needed, this value is quite good when compared to the positive control, doxorubicin with IC₅₀ 0.93 μg/mL. Although it has not been able to exceed doxorubicin, flavonoids from E. cinereum have good potential to be used as an alternative therapy or additional therapy for cancer. In addition, it also proves scientifically the empirical use in society. Moreover, the culture of the Indonesian people used traditional medicine as additional treatment or a companion to conventional medicine. Consuming this plant in the right way can improve cancer healing in Indonesia.
Identification was done by RP-HPLC with PDA detector and TLC. Chromatogram of isolate (flavonoid) shows a single peak and has a wavelength of 273.5 nm and 376 nm, which is very identical to flavonoids. At this wavelength, it is suspected that the flavonoid is a type of flavonol. The results of identification by TLC also showed that the isolate was a flavonoid with a light yellow.

Flavonoid compounds have been widely used as a source of antioxidants that contribute to preventive and curative in cancer. Previous studies have proven that flavonoid compounds in vitro and in vivo can inhibit the process of carcinogenesis through various mechanisms of action including triggering apoptosis, for example, the flavonoid compounds quercetin and calycopterin have been shown to inhibit breast cancer cells. The antiproliferative effect of the flavonoid calycopterin occurs in a dose-dependent manner with the mechanism of inhibition of colony formation activity, decreased intracellular migration, and increased expression of apoptosis-related genes (Bax genes) in MCF-7 cells. Flavonoid compounds can also bind to the core estrogen receptors ERα and ERβ.

In previous studies, the genus *Eriocaulon* was reported to contain compounds that have been shown to have cytotoxic activity against cancer cells with IC₅₀ 7.65 μg/mL. The flavonoid compounds that have been identified include (2S)-3',4'-methylenedioxy-5,7-dimethoxyflavan, hispidulin, quercetagetin, and gossypetin.

**CONCLUSIONS**

Flavonoid from *E. cinereum* R. Br showed cytotoxic activity against MCF-7 cells with IC₅₀ 7.28 ± 6.75 μg/mL. This also
proves that scientific search results are in line with empirical used. *E. cinereum* R. Br should be developed as adjuvant therapy and consume as traditional medicine.

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**Conflicts of interest**

There are no conflicts of interest.

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