Cytotoxic activity of erypogein d from erythrina poeppigiana (leguminosae) against cervical cancer (HeLa), breast cancer (MCF-7) and ovarian cancer (SKOV-3) cells

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Abstract. Cancer is the uncontrolled growth of abnormal cells and continues to divide rapidly in the body. Current anticancer treatment usually causes many side effects. Natural products are then explored to be new alternatives for cancer treatment. Flavonoids have been known to possess medicinal properties, including anticancer. This study was performed to observe the cytotoxic activity of isoflavone compound, erypogein D from Erythrina poeppigiana, toward cervical cancer (HeLa), breast cancer (MCF-7) and ovarian cancer (SKOV-3) cells. The cytotoxic activity of erypogein D was tested using MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxyme-thoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) assay. The percentage of cell mortality was calculated and the IC50 was analyzed using probit analysis. The result showed that cytotoxic activity of the erypogein D against HeLa, SKOV-3, and MCF-7 cells had an IC50 value 225, 70.74, and 30.12 µM, respectively. Based on IC50 value can be concluded that erypogein D is the most cytotoxic to breast cancer MCF-7 cell. However the cytotoxic activity of erypogein D toward MCF7 is moderate.

1. Introduction
Cancer is the second cause of death after cardiovascular diseases in the world. There were around 8.2 million of people died because of cancer and about 14.1 million of new cancer cases were documented in 2012 [1]. Particularly in Indonesia, there were about 0.1% of total people suffering from cancer with 36.7% of mortality rate in male and 63.3% in female [2]. Chemotherapy is usually used for cancer treatment. However, chemotherapeutic treatments are reported to generate various kinds of toxicities such as cardiotoxicity [3], cardiac toxicity [4], renal toxicity [5], and myelotoxicity [6]. The toxicity of chemotherapeutic drugs sometimes creates a significant problem in the treatment of cancer using allopathic or established medicine.

Plant-derived products has been recently studied as new alternative in cancer treatment. Many compounds from medicinal plants with potential anticancer activities have been reported [7]. Phenolic acids, flavonoids, stilbenes, and lignans are the most abundant polyphenols in plants, out of which flavonoids and phenolic acids account for 60% and 30%, respectively, of dietary polyphenols. They possess medicinal properties due to its ability to interact with number of cellular targets, such as anti-
oxidant and free-radical scavenger activities also the anti-inflammatory, antiviral, and especially anticancer [8-10].

The Genus of Erythrina belong to Leguminosae family is higher plant and famous medicinal plant widely distributed in tropical and subtropical region of the world. Extracts of the leaves, stem bark, and roots of Erythrina have been used as a folk medicine for anthelmintic, cancer, malaria, and inflammatory processes. Previous phytochemical analysis of this plant has revealed the presence of erythrina alkaloids [11,12], and isoflavonoids [13]. In particular, it is rich in isoflavones [14], coumestans [15], and arylbenzofurans [16]. Erypogein D is an isoflavanone compound (Fig.1) isolated from stem bark of Erythrina poeppigiana [17] that has never been studied for cytotoxic activity. Thus, in this study, we reported the cytotoxic activity of erypogein D against cervical cancer (HeLa), breast cancer (MCF-7) and ovarian cancer (SKOV-3) cells.

2. Methods

2.1. Plant Materials
The stem bark of E. poeppigiana were freshly collected in September, 2016, in Bandung District, West Java, Indonesia. The plant was identified by a staff at the Laboratory of Plant Taxonomy, Department of Biology, Padjadjaran University, Bandung, Indonesia, and a voucher specimen has been deposited at the herbarium.

2.2. Extraction and isolation compound
The erypogein D was isolated from the stem bark of of E. poeppigiana in the Organic Chemistry Laboratory, Padjadjaran University, Jatinangor, Bandung, Indonesia. A methanol extract of E. poeppigiana was dissolved in water and partitioned between n-hexane and ethyl acetate. The ethyl acetate fraction was further separated through a combination of column chromatography on Kieselgel 60 to afford erypogein D compound was identified by comparison of their spectroscopic data with reported values [17].

2.3. Cell Culture (MCF7, SKOV-3, and HeLa cells)
The MCF-7, SKOV-3 and HeLa cells were used for cytotoxic screening of the tested compounds. The MCF-7 (ATCC, HTB-22), SKOV-3 (ATCC, HTB-77) and HeLa (ATCC, CCL-2) cells were obtained from Aretha Medika Utama Biomolecular and Biomedical Research Center, Bandung, Indonesia. The MCF-7 and HeLa cell lines were maintained in DMEM (Gibco, 1195-065), 10% fetal bovine serum (FBS) (Gibco, 10270), and 1% Abam (Gibco, 15240-062), while SKOV-3 cell lines were maintained in McCoy’s BA (Gibco, 16600-082), 10% fetal bovine serum (FBS) (Gibco, 10270), and 1% Abam (Gibco, 15240-062). The cell lines were maintained at 37 °C in a 5% CO2 atmosphere with 95% humidity. After 24 hours of incubation, the number of viable cells was counted using a haemocytometer with Trypan blue staining [18].

2.4. Cytotoxic Assay
Cytotoxic activity of the erypogein D on the treated cells were determined using the MTS colorimetric assay modified method. The treated cells culture (90 µL) at density of 104 cells/well were distributed in 96-wells plates. Ten microlitres of the erypogein D at various concentration (µg/mL) was added to each well. Culture medium alone was served as negative control. The plate was then incubated for 24 h at 37 °C in a 5% CO2. The mixture was further added with 20 µL of MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxyme-thoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium), incubated for 4 hours at 37 °C in 5% of CO2. The absorbance was read at 490 nm wavelength [18].

The cell growth inhibition was determined with the equation 1:

\[
\text{% of cell growth inhibition} = \frac{\text{OD of control cell} - \text{OD of treated cell}}{\text{OD of control cell}} \times 100\%
\]  

(1)
% of cell growth inhibition = (OD of control cell - OD of treated cell)/(OD of control cell) x 100%

Inhibitory concentration (IC50) were determined using probit analysis. The IC50 values was obtained from three independent experiments.

2.5. Statistical Analysis
Data of the IC50 values and SI were presented as mean ± standard deviation (SD) and were analyzed using Duncan HSD Post Hoc Test. A p value less than 0.05 was considered as statistically significant.

3. Results and Discussion

3.1. Characteristics of Erypogein D
Erypogein D as yellow oil. Erypogein D with another name is (S)-5,4′-dihydroxy-7,2′-dimethoxy-5′-(3′-methylbut-2′-enyl) isoflavanone; [α]D -1.82 (c26.00g/100cm3, MeOH); IR (KBr): 3400, 2900, 1639, 1460, 800 cm-1; UV/Vis λmax (MeOH) nm (log ε): 287 (4.08), 203 (4.52); 1H NMR (500 MHz) and 13C NMR (125 MHz), acetone-d6,Table 5; HR-TOFMS ES+ spectrum showed [M+H]+ 385.1646, calcd m/z 385.1651 [17].

![Figure 1. Chemical Structure of Erypogein D](attachment:image.png)

3.2. Cytotoxic Activity Erypogein D against cervical cancer (Hela), breast cancer (MCF-7) and ovarian cancer (SKOV-3) cells.
As shown in Table 1, erypogein D had the the lowest IC50 in breast cancer MCF-7 (30.12 µM) which indicated highest cytotoxicity compared to ovarian cancer SKOV-3 and cervical cancer HeLa cells. Erypogein D is the most selective to breast cancer MCF-7 cell, however the cytotoxic activity of erypogein D is moderate.

| Cells  | IC50 (µM) |
|--------|-----------|
| MCF-7  | 30.12     |
| SCOV-3 | 70.74     |
| HeLa   | 225       |

Flavonoids that has a variety of substituents on A ring had been investigated for antiproliferative activity against MCF-7 human breast cancer cells. The presence of hydroxyl and methoxy groups attached to the flavonoids may have an effect on the activity of MCF-7 breast cancer [19].

Flavonoid compounds such as erythraddison I, erythraddison II, erythraddison III, and erythraddison IV of Erythrina addisioniae showed cytotoxic activity against breast cancer cells MCF-7 with IC50 values >30, 17.4±1.1, 4.6±0.3, and 13.8±1.8, respectively [20].

Previous study showed that, flavonoids can inhibit the growth of breast cancer MCF-7 cell. In the presence of flavonoid, the normal cells grew normally, whereas the breast cancer cells underwent a change in morphology [21].

Based on the Figure 2, the morphological appearance of MCF-7 cells treated with erypogein D showed the decreasing of cell number, which indicate the effects of treatment toward MCF-7 cells growth. In the Figure 3 and 4, we can see that the morphological appearance of SKOV-3 and HeLa
cells also showed the decreasing of cell number compare to control cells Its indicate that treatment with erypogein D influence the cell growth.

The result of present study suggests that the erypogein D might be a promising agent for anticancer treatment. Flavonoids are a diverse and historically important family of natural products. Various plants and species containing flavonoid derivatives have demonstrated their potency as preventive and therapeutic agents [22]. Among the range of pharmacological activities of flavonoids, their anticancer activity is of particular interest [23]. The anticarcinogenic effects of flavonoids have been observed in vitro and in vivo, in which the compounds have been found to inhibit and protect against various stages of cancer processes, including proliferation, inflammation, angiogenesis, invasion and metastasis [24]. These actions are attributable to their polyphenolic structure [25].

**MCF-7**

![MCF-7 images](image1)

**Figure 2.** Morphological appearance of MCF-7 cells with no treatment (a) and cells with treatment at various concentrations: (b) 1.17, (c) 9.38, and (d) 37.50(µM). Scale bar: 100 µM. The normal cells relatively has higher density than treated cells.

**SKOV3**

![SKOV3 images](image2)

**Figure 3.** Morphological appearance of SKOV-3 cells with no treatment (a) and cells with treatment at various concentrations: (a) 1.17, (b) 9.38, and (c) 37.50 (µM). Scale bar: 100 µM. The normal cells relatively has higher density than treated cells.
HeLa

Figure 4. Morphological appearance of HeLa cells with no treatment (a) and cells with treatment at various concentrations: (b) 1.17, (c) 9.38, and (d) 37.50 (µM). Scale bar: 100 µM. The normal cells relatively has higher density than treated cells.

4. Conclusion
Erypogein D compounds from Erythrina poeppigiana showed the highest cytotoxic activity against breast cancer MCF-7 cell (IC50 30.12 M) compared with cervical cancer HeLa and ovarian cancer SKOV-3 cells in vitro by MTS assay method. Erypogein D has a category of moderate cytotoxic activity.

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