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Research Article

CYTOTOXIC ACTIVITY OF α-ERYTHROIDINE FROM THE STEM BARK OF *ERYTHRINA POEPPIGIANA* AGAINST MCF-7 BREAST CANCER CELL LINE

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ABSTRACT

The stem bark of *Erythrina poeppigiana* from the Leguminosae family has been used for relieving fever, infections, and inflammation. This plant contains erythrinan alkaloids as a major compound. In the course of our research for the cytotoxic compound from *Erythrina* genus, the methylene chloride (CH_2Cl_2) extract showed moderate activity against MCF-7 breast cancer cell line. The CH_2Cl_2 extract was separated by several column chromatography methods to yield α-erythroidine, based on spectroscopic comparison with previously published. α-Erythroidine showed cytotoxic activity against MCF-7 cell linewith IC_{50} value 11.60 µg/mL.

Keywords: *Erythrina poeppigiana*, α-erythroidine,MCF-7 cells, anticancer

INTRODUCTION

Breast cancer still to be most commonly suffered by women around the world and the number of new cases grows continually[1]. Available treatments that use anticancer compounds (taxanes, vinca alkaloid, camptothecin derivate, topotecan, etoposide, antra cyclins) take a long period and may endanger the other normal cells[2]. The main problem is the growing resistance of cancer cells against anticancer drugs[3]. Naturally derived compounds be major attention as alternative medication which shown good effects on the patient[4]. The plants provide secondary metabolites potentially for bioactive compounds, the one is *Erythrina poeppigiana*.

Erythrina poeppigiana is locally known as "dadap belendung" in Indonesia. The leaves and barks have used in folk remedies for the treatment of joint pain, fever, inflammation, and microbial infections[5,6]. Previous phytochemical studies have stated the presence of eryhtrinan alkaloids and isoflavonoids in several parts of this plant[7-9]. Prior analysis has revealed that the wood of *E. poeppigiana* gave 8-oxo-α-erythroidine epoxide, 8-oxo-α-erythroidine, and 8-oxoerythraline epoxide [10]. The stem bark of *E. poeppigiana* contain α-erythroidine, β-erythroidine, 8-oxo-α-erythroidine, and 8-oxo-β-erythroidine which shown the estrogenic activity, bind the estrogen receptor alpha and beta (ERα and β), owned by MCF-7 cell line[6].

As part of our research project focusing on erythrinan alkaloid which shown cytotoxicity, investigations of the stem barks were performed, led to obtaining α-erythroidine. This alkaloid exhibits cytotoxic activity against MCF-7 cell line. The isolation and structural elucidation were described in this study.

MATERIAL AND METHODS

General Experiment Procedures

UV spectra were recorded on UV-1800 Shimadzu spectrophotometer. IR spectra were obtained on One Perkin Elmer Spectrum FTIR spectrometer, HRTOFMS data were recorded on Waters QTOF Xevo spectrometer. ^1H, ^{13}C, DEPT, HSQC, and HMBC data were recorded on Agilent 500 MHz NMR spectrometer operating at 500 MHz for ^1H and 125 MHz for ^{13}C nuclei, respectively. Solvent peaks were used as a reference standard. Column chromatography (CC) was performed on silica gel G60 (70-230 mesh, Merck). Fractions were monitored by TLC on silica gel plates (GF_{254}, Merck), spots were visualized under UV lamps (VilberLourmat at λ 254 and 365 nm) and Dragendorff's reagent spray for alkaloid guiding.

Plant Material

Stem barks of *E. poeppigiana* were collected in January 2018 in Bandung District, West Java, Indonesia. The plant was identified by the staff at Plant Taxonomy Laboratory, Department of Biology, Universitas Padjadjaran, West Java, Indonesia. The sample was deposited in the herbarium. Voucher specimen number: 540/HB/02/2017.

Extraction and Isolation

The stem barks (2.6 kg) were ground to powder and macerated with CH_3OH (3×24 hours) at room temperature to give crude extract, which acidified with 2 M HCl to pH 2. After fractionated by CH_2Cl_2 (1:1) to exclude non-alkaloids, the acidic aqueous phase was increased to pH 10 by adding NH_3 25%, followed by a second fractionation with CH_2Cl_2 to obtain CH_2Cl_2 soluble extract (3.89 g). The extract was separated using CC silica gel and

eluted using $CHCl_3$: EtOAc (100:0 – 90:10) 5% stepwise to afford four major fractions (A – D). Fraction B (854 mg) was subjected to CC with isocratic $CHCl_3$: EtOAc (9:1) to obtain three fractions (B.1-3) monitored on TLC. Fraction B.3 (284.6 mg) was further purified using CC with n-hexane: EtOAc: CH_3OH (2.5:7:0.5) in the isocratic system, to give α-erythroidine (10.6 mg).

Cell Culture

The MCF-7 breast cancer cell line was used for cytotoxic assays, obtained in a frozen-phase under liquid nitrogen and thawed immediately at 37°C from Parasitology Laboratory Universitas Gadjah Mada (UGM). The MCF-7 cells were suspended in Dulbecco's modified Eagle's medium (DMEM, Gibco) supplemented with 10% fetal bovine serum solution (Gibco), 2% penicillin-streptomycin, 0.5% fungizone (Gibco) and added by DMEM media 100%, incubated for 2–3 days at 37°C in 5% CO_2 incubator. After incubation, the number of viable cells was counted using the hemocytometer[11].

Cytotoxic Assay

The cytotoxic against MCF-7 cells was performed using the 3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay. In summary, the treated cell culture (1×10^4) were distributed in 96-well plates and incubated in 5% CO_2 incubator at 37°C overnight. α-Erythroidine at various concentration (500; 250; 125; 62.5; 31.25; 15.625; 7.8125; 3.90; 1.95; 0.98 µg/mL) was added to each well and incubated for 24 hours. The culture medium was served as the negative control. At the end of incubation, medium and sample were washed by phosphate bovine serum. Each well was added with 100 µL of 0.5 mg/mL MTT, incubated for 4–6 hours at 37°C in 5% CO_2 incubator. The absorbance was read at 595 nm wavelength.

The cell growth MCF-7 inhibition was determined with the equation:

Viable cell percentage = [(Absorbance of treated cell – Absorbance of medium) / (Absorbance of control cell – Absorbance of medium)] × 100%

The cytotoxic activity was expressed with IC_{50} value which analyzed using linear regression equation[12].

RESULTS AND DISCUSSION

Structure Elucidation of α-Erythroidine

The CH_2Cl_2 soluble extract of the stem bark of *E. poeppigiana* was separated by a combination of chromatographic methods, gave α-erythroidine (Figure1), on the basis of spectroscopic comparison with published literature[8].

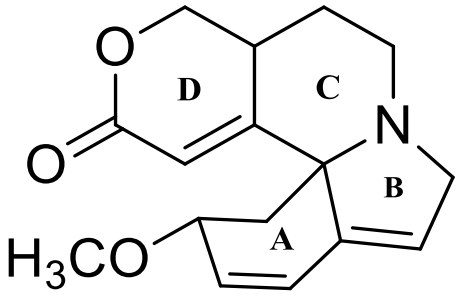


Figure 1: Structure of α-erythroidine

α-Erythroidine was isolated as a brownish-yellow oily substance. The compound showed molecular ion peaks at m/z274.1370 $[M+H]^+$(calcd 274.1443 for $C_{16}H_{20}NO_3$) using HR-TOFMS. The UV spectrum revealed the maximum absorption at 223.6 nm, indicative of conjugated dienes, seems the conjugated diene is of the type shown[13]. The IR spectrum showed a carbonyl group at δ-lactone (1711 cm^{-1}).

The ^1H NMR and HSQC spectrum displayed the typical conjugate olefin signals at H-1, H-2 and H-7 (δ_H 6.47,5.69, 5.85), respectively, indicated A and B-rings of conjugated dienoid type erythrinan alkaloids, an olefinic singlet proton at H-14 (δ_H 5.94) revealed a diene structure at D-ring, three methylene groups at H-8,H-10, H-17 [δ_H 3.55 (1 H, dd, *J* = 14.9, 3.1 Hz), 3.67 (1 H, d, *J* = 14.9 Hz), 2.94 (1 H, dt, *J* = 15, 3.85 Hz), 3.26 (1 H, dd, *J* = 15, 7.95 Hz), 4.09 (1H, dd, *J* = 11.4, 2.75 Hz), 4.48 (1 H, dd, *J* = 11.4, 5.65 Hz)] evidencedthis proton to be in one environment (*J* = 11-15 Hz), and a methoxy group [δ_H3.38 (3H, s)].

The ^{13}C NMR and DEPT spectra showed 16 carbons, there are five methylenes (including an oxygenated methylene δ_C 71.2, 56.5 and 44.1 indicated two C-N signals), six methines, one methoxy (δ_C 56.6), and four quaternary carbons (including two olefinics). The HMBC correlation (Figure 2) confirmed the A and B-conjugated rings, H-1 (δ_H 6.47)/ C-3 (δ_C 76.9) and C-5 (69.0), H-8 (δ_H 3.55)/ C-1 (δ_C124.5), C-6 (δ_C 141.3). An olefin position was observed, correlation H-4 (δ_H 1.61, 2.78)/ C-13 (δ_C 164.2) and C-14 (δ_C 133.0) revealed olefin carbon at C-13 and C-14 D-ring. The presence of a methoxy moiety at C-3 was further confirmed by the HMBC correlation observed from methoxy protons H-18 (δ_H 3.38) to C-3 (δ_C 76.9) indicated that C-3 was substituted with a methoxy group. The HMBC correlation of H-17 (δ_H 4.09, 4.48) to C-15 (δ_C 166.9) and C-13 (164.2) revealed a carbonyl group at quaternary carbon C-15. Methylene proton of H-17 correlated with carbon (δ_C 71.2), typical for oxygenated carbon, was further supported by its HMBC spectrum, H-17 to C-15 and C-12 (δ_C 31.7) indicated an oxygen atom at position 16. Thus, the structure of the isolated compound was determined as α-erythroidine, compared with prior literature[8].

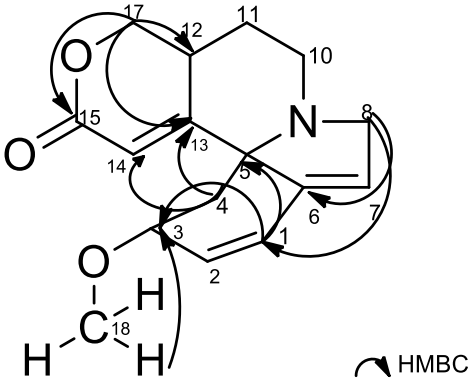


Figure 2: Structure α-erythroidine with HMBC Correlation

Cytotoxic Activity α-Erythroidine against MCF-7 Breast Cancer Cell Line

α-Erythroidine showed cytotoxic activity against the MCF-7 cell line at a concentration of 11.60 µg/mL. This compound was isolated from CH_2Cl_2 soluble extract which had activity against the MCF-7 breast cancer cell at 53 µg/mL, as given in table below.

Table 1: IC_{50} values of CH_3OH and CH_2Cl_2 soluble extract against MCF-7 breast cancer cells

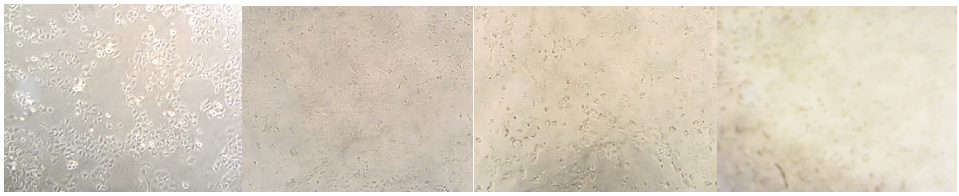
Sample	IC_{50} (µg/mL)
CH_3OH extract	575.08
CH_2Cl_2 soluble extract	53.00
α-Erythroidine	11.60
Cisplatin	5.30

This finding means that either this alkaloid act synergistically as a group with its extract, which showed moderate toxicity against the MCF-7 cell line.

The previous study revealed that α-erythroidine has estrogenic activity, binds the estrogen receptor alpha (ERα) with IC_{50} value 57.3 µM[8]. The MCF-7 cell line expresses substantial levels of ER, like invasive human breast cancer that express ER[14]. The role of ERα is to promote proliferation and migration in the breast cancer cell, if it binds to estrogenic compound, the function of ERα could be blocked[15].

Erythrinan alkaloid has several groups, depending on D-ring modify. The erythra- group; 10, 11-dioxoerythratidine from the leaves of *E. variegata* has a strong activity against *P. falciparum* strain K1 and T47D breast cancer cell with IC_{50} values 3.2 and 1.0 µg/mL, respectively[16].

The morphological of MCF-7 cells treated with α-erythroidine showed a reduction in the cell number, indicated the decreasing MCF-7 cells growth influenced by treatment with α-erythroidine at 15.625 µg/mL (Figure 3). The role of α-erythroidine has an effect on the cell death, the increasing concentration of this alkaloid, would increase the MCF-7 cell's death.



(a) (b) (c) (d)

Figure 3: Morphological appearance of MCF-7 cells with no treatment (a) and with treatment at various concentrations: (b) 0.98, (c) 3.90, and (d) 15.625 µg/mL. Scale bar: 100x. The normal cells relatively have a higher density than treated cells

This result suggests that α-erythroidine might to be a promising agent for anticancer treatment. Erythrinan alkaloids are distributed in the *Erythrina* genus and have special pharmacological properties, the one is an anticancer activity[17]. Various substituents attached to D-rings attribute to the cytotoxic activity of the alkaloids[18]. The presence of α, β-unsaturated carbonyl group (Ring D) in α-erythroidine was considered to be the attribute to its cytotoxicity.

CONCLUSION

In this study, α-erythroidine was isolated from the stem bark of *E. poeppigiana*. This compound has moderate cytotoxic activity against MCF-7 breast cancer cell line with IC_{50} value 11.60 µg/mL.

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