Isoquinoline Alkaloids from *Erythrinapoeppigiana* (Leguminosae) and Cytotoxic Activity Against Breast Cancer Cells Line MCF-7 In Silico

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Abstract. *Erythrinapoeppigiana* (Leguminosae) is a higher plant that has been used as a folk for the treatment of infection, fever, and inflammation. In the course of our continuing search for novel cytotoxic compounds from genus *Erythrina*, the methanol extract of *E. poeppigiana* showed a significant cytotoxic activity against breast cancer cells line MCF-7 in silico. The compounds in methanol extract of the *E. poeppigiana* was separated using a bioassay-guided fractionation. By using a cytotoxic activity to follow separation, the methylene chloride was separated by several column chromatography techniques on silica gel and ODS to yield three active compounds (1-3). The chemical structures of active compounds were determined on the basis of spectroscopic evidence and comparison with those identical compounds that previously reported and identified as a 10,11-di hydroxyerysodine (1), 6,7-dihydro-17-hydroxyerysotrine (2), 6,7-dihydro-11-methoxyerysotine (3). Compounds (1-3) showed cytotoxic activity inhibits EGFR 2 against breast cancer cell line MCF-7 in silico molecular docking method with bond Gibbs free energy (ΔG) (kcal/mol) and inhibition constants (Ki) (nM) of value (-8.61121, 4.84x10^-7); (-8.1145, 1.12x10^-6); and (-7.3394, 4.14x10^-6), respectively.

1. Introduction
Breast cancer is the most commonly diagnosed invasive non-skin malignancy and second leading cause (after lung cancer) of cancer related deaths in the women. Both epidemiological and laboratory studies show that environmental and behavioural factors are more important than genetic factors in determining overall cancer frequency among populations. Currently, systemic cytotoxic chemotherapy approaches, controlling and treating breast cancer, are being used which are not only less effective but are also non-selective and highly toxic to normal tissues [1].

Recently, the acceptance of traditional medicines as an alternative form of healthcare has increased among all socio-economic groups of the population in Indonesia. For these reasons, medicinal plants have become the focus of intense study, in term of validation of their traditional uses, through the determination of their actual pharmacological effects [2]. Recently, the attention has been focused on medicinal plants to provide new anti-cancer agents. *Erythrinapoeppigiana* (Leguminosae) is a famous medicinal plant widely distributed in tropical and subtropical region of the world. This plant is locally known as “dadapbelendung” in Indonesia and the leaves of *E. poeppigiana* are used as folk medicinal to treatment infection, inflammation, and fever. Previous studies have shown that the stem bark of *E. poeppigiana* contain isoquinoline, α-erythroidine, 8-oxo-α-erythroidine, and 8-oxo-β-erythroidine alkaloid [3,4]. As part of our continuing search for novel anti-cancer compounds from *E.
*poeppigiana* plant, the isolation alkaloid and test the cytotoxic activity against breast cancer cell line MCF-7 that its mechanism of action inhibits the Epidermal Growth Factor Receptor 2 (EGFR 2) by molecular docking in silico method has been described during the present investigations. In short of our experimental procedure, we extract and isolate alkaloid, using common methods. Detailed method can be found in many references [5].

2. **Experimental Method**

2.1. **Plant Materials**

The leaves of *E. poeppigiana* were freshly collected in September, 2014, in Bandung District, West Java, Indonesia. The plant was identified by a staff at the Laboratory of Plant Taxonomy, Department of Biology, Universitas Padjadjaran, Bandung, Indonesia, and a voucher specimen has been deposited at the herbarium.

2.2. **Extraction and isolation compounds (1, 2, and 3).**

The dried leaves (2.6 kg) of *E. poeppigiana* were extraction of methanol acidified with 1% hydrochloric acid to a pH of 2-3. Furthermore, fraction of acidic water was basified with ammonium hydroxide until pH 9-10 and solvent methylene chloride (1:1). Methylene chloride fraction (11.2 g) was separated using column chromatography silica gel G60 (70-320 mesh) with an eluent of chloroform: methanol (9.8: 0.2) in isocratic, obtained four fractions (F1-4) positive alkaloid. Fraction F1 is purified using column chromatography silica gel with chloroform: methanol (9:1) in isocratic system, obtained four fractions (F1A-D). F1B fraction was purified using silica gel column chromatography with chloroform: methanol (0.25%), obtained three fractions (F1B1-3). F1B2 fraction was separated using preparative thin layer chromatography with eluent ethyl acetate: acetone (9:1), obtained compound 1 (4.5 mg). F2 fraction was separated using silica gel column chromatography with chloroform: methanol (2%), obtained four fractions (F2A-D). F2A fraction separated using preparative thin layer chromatography with methylene chloride eluent: chloroform: methanol (1: 0.9: 0.1), obtained compound 2 (8.1 mg). F2B fraction separated using silica gel column chromatography column with isocratic solvent methylene chloride: chloroform: methanol (1:0.9:0.1) obtained four fractions (F2B1-3). F2B2 fraction separated using preparative thin layer chromatography with solvent methylene chloride: methanol (9:1), obtained compound 3 (10.4 mg).

2.3. **Preparation of Ligands**

Ligands are drawn in 2D using software MarvinSketch 5.2, and then do the geometry optimization protonated ligand with a pH of 7.4, save in the form .mrv. Searching for the determination of confirmation structure and save as a file in the form of pdb and moles [6].

2.4. **Validation of Docking Method**

ArgusLab program validated to get a reliable method. Receptors that are used are obtained from the tuberculosis receptor protein banks data. Docking method is used to be good if Root Mean Square Deviation (MRSD) value less than or equal to 2 [7].

2.5. **Docking Using ArgusDock**

Docking compound with epidermal growth factor receptor 2 (EGFR 2) use the software ArgusLab. In the process of docking used each grid box receptors that are valid on previous validation process.

3. **Result and Discussion**

3.1. **Characteristics of Compounds (1-3)**

10,11-dihydroxyerysodine (1): yellow oily; Uvλmax (MeOH) nm 237 (ε 3846 ); 278 (ε 1009 ); IR (KBr) Vmax 3400, 2923, 1641, and 1382 cm⁻¹; HR-TOFMS [ M-H ]⁺ m/z ; ( 298.1017 ); ¹H-NMR (CD3OD): δ8 4.12 (H, d, 5), 7.55 (1H, d, 5), 3.58 (1H, m), 2.14 (1H, m), 2.12 (1H, m), 5.32 (1H, bs ), 4.54 (1H,m), 4.40 (1H, m), 6.80(1H,d, 5), 6.79(1H,d, 5), 7.24 (1H, s), 7.45(1H, s ), 3.99 (3H, s),
3.73(3H, s), 4.02 (1H,s); ^1^C- NMR (CD3OD): \( \delta \) c121.9, 130.4, 73.3, 42.7, 64.8, 140.9, 119.4, 37.8, 120.5, 113.1, 127.1, 129.4, 106.2, 148.4, 148.3, 108.6, 56.2, 56.3 ppm.

6,7-dihydro-17-hydroxyerysotrine (2)[8].

6,7-dihydro-11-methoxyerysotrine (3): white powder; 1H-NMR (CD3OD): \( \delta \) H 3.81 (3H, s), 3.50 (3H, s), 3.74 (3H,s), 3.49 (3H,s), \( \delta \) H 6.66 (1H, d, \( J \)=10, H-1), 6.56 ppm (1H, d, \( J \)=10, H-2), 3.81(3H, s, H-3), 1.59 (1H, brs, H-6), 2.89 (2H, m, H-7), 3.17 (1H, m, H-8), 3.83(1H, m),3.28 (2H, m, H-10), 3.55 (1H, m, H-11), 3.49 ppm (3H, s, 11-OCH3), 3.74 (3H, s, 15-OCH3), 3.50 ppm (3H, s, 16-OCH3).

3.2. Docking of Compounds (1-3)

This program should be added hydrogen ArgusLab receptors, because receptor in pdb is not hydrogen bonds. An 3W32 native ligand and a ligand copy docked with EGFR2 reseptor using ArgusDock method values 1.917605 A RMSD obtained using the grid method box X = 20.75 ; Y = 14:25 , and Z = 17:25 with a grid resolution of 0.4 A on condition of flexible ligands. RMSD smaller value than 2 indicates that a valid method of docking, so it can be used for docking of compounds 1, 2, and 3. Results docking compounds 1, 2, and 3 of the EGFR 2 receptor compared with canertinib compounds which are anticancer drugs work on the receptor EGFR 2. Results docking form of the Gibbs free energy and inhibition constants (Table 1).

| Compounds                           | \( \Delta \text{G} \) (kcal/mol) | \( \text{Ki} \) (nM) |
|-------------------------------------|----------------------------------|---------------------|
| 10,11-dihydroxyerysodine (1)        | -8.61121                         | 4.84 x 10^-7       |
| 6,7-dihydro-17-hydroxyerysotrine (2)| -8.11457                         | 1.1 x 10^-6        |
| 6,7-dihydro-11-methoxyerysotrine (3)| -7.3394                          | 4.14 x 10^-6       |
| Canertinib                          | -8.11505                         | 1.1 x 10^-6        |

Results of docking (Table 1) showed that compounds 1, 2, and 3 have a Gibbs free energy which is almost equal to the compound canertinib. Compounds 1, 2, and 3 can be predicted to have similar activity such as anticancer canertinib working on EGFR 2 receptor. The lower of value a Gibbs free energy released during the interaction between compounds 1, 2, and 3 with a receptor showed that stability and strength of interaction of non-covalent bonding cause easily get into cells and interfere with DNA replication or the metabolic process to cell death.

\( \Delta \text{G} \) value is proportional to a constant value inhibition. Value inhibition constants (Ki) illustrate the affinity of compounds (1, 2, and 3) and decomposition. The smaller of Ki value, the equilibrium of reaction tends towards formation of compound-receptor complexes (Purnomo, 2013). The complex compound-receptor binding affinity is said to have a good if it has a Ki value in the nanomolar scale. Docking results showed that compounds (1, 2, and 3) have an estimated value of Ki in the nanomolar scale. The smaller value of the inhibition constants, reaction equilibrium tends toward complex formation of compounds 1, 2, and 3 with a receptors. The results showed that a docking 3D visualization of the interaction between all the compounds 1, 2, and 3 with EGFR 2 reseptor (Figure 2). Compounds 1, 2, and 3 through the docking process showed the properties as inhibitors of EGFR 2 receptor. Epidermal growth factor (EGF) receptor (EGFR) has most an important role in the growth and development of breast cancer MCF-7 [9].
Figure 2 showed that compound 1 (purple), compound 2 (yellow), compound 3 (light blue) and a canertinib (green) through a process of docking in the area would bind of target compound (black) which are known to have properties as an inhibitor of EGFR 2.

Figure 2. Visualization of an interaction between compounds 1 (purple), 2 (yellow), 3 (blue pale), and canertinib (green) with EGFR 2 receptor.

4. Conclusion
Three alkaloids isoquinoline: 10,11-dihydroxyerysodine (1), 6,7-dihydro-17-hydroxyerysotrine (2), and 6,7-dihydro-11-methoxyerysotrine (3) isolated from leaves of *E. poeppigiana*. Molecular docking showed that compounds 1, 2, and 3 have a Gibbs free energy which is almost equal to canertinib. Compounds (1-3) showed cytotoxic activity inhibits EGFR 2 against breast cancer cell line MCF-7 in silico using molecular docking method with bond Gibbs free energy (ΔG) (kcal/mol) and inhibition constants (Ki) (nM) of value (-8.61121, 4.84x10^{-7}); (-8.1145, 1.12x10^{-6}); and (-7.3394, 4.14x10^{-6}), respectively. Compounds 1,2 , and 3 can be predicted to have similar activity such as anticancer canertinib working on EGFR 2 receptor. Compounds 1,2, and 3 are predicted to have a cytotoxic activity against cell MCF-7 breast cancer using molecular docking method.

5. References
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