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**POTENTIAL OF DADAP AYAM (*Erythrina variegata*) PLANT
AS HERBAL MEDICINE**

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ABSTRACT

Introduction: Traditional medicines originating from plants have been widely used as an alternative therapy and extensive research have identified several bioactive compounds of the plants. In Indonesia, *Erythrina variegata* (Leguminosae) plants have been used for medication to many diseases including anthelmintic, anticancer, antimalaria, and antifertility. **Objective:** In the course of our continuing search for novel bioactive compounds from Indonesian *Erythrina* plants, we isolated and described several bioactive compounds such as anticancer, antimalaria, and antifertility from *E. variegata*. **Method:** Activity assay in vitro against breast cancer cell T47D using the Sulforhodamine B (SRB) method, activity assay in vitro against antiplasmodium using lactatedehydrogenase (LDH) method, and antifertility activity on spermatozoa *Rattus norvegicus*. Their chemical structures were determined based on spectroscopic evidences and comparison with related compound previously reported. **Result:** Extract and compounds of *E. variegata* showed anticancer in vitro against breast cancer cell T47D, antiplasmodial in vitro K1 and 3D7 strain parasites, and antifertility on spermatozoa *R. norvegicus* with middle and high activity. Besides that, the toxicity assay of it was also conducted using white mice to show the safety grade of the plant. **Conclusion:** This results strongly suggested that *E. variegata* is a promising sources as herbal medicine of anticancer, antimalaria, and antifertility agents.

Keywords: Anticancer, antifertility, antimalaria, *Erythrina variegata*, Leguminosae

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**POTENSI TANAMAN DADAP AYAM (*Erythrina variegata*)
SEBAGAI OBAT HERBAL**

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ABSTRAK

Pendahuluan: Pengobatan tradisional menggunakan tumbuhan sudah digunakan secara luas sebagai pengobatan alternatif dan beberapa penelitian medis telah menemukan zat aktif yang berguna yang terkandung dalam tanaman. Tanaman dadap ayam sudah sering digunakan sebagai obat antelmintik, kanker, malaria, dan sebagai kesuburan di Indonesia. **Tujuan** penelitian ini adalah untuk menindaklanjuti hasil penelitian dengan ditemukannya zat aktif yang terkandung dalam tanaman dadap ayam Indonesia. Beberapa kandungan senyawa aktif tanaman dadap ayam ini diuji efeknya sebagai anti kanker, anti malaria, dan anti kesuburan. **Metode:** pengujian secara in vitro terhadap sel kanker payudara T47D menggunakan metode sulforhodamin B, pengujian terhadap antiplasmodium menggunakan metode laktatdehidrogenase, dan anti kesuburan diteliti pada aktifitas spermatozoa *Rattus norvegicus*. Struktur kimia ditentukan menggunakan spektroskopik. **Hasil:** Ekstrak *Erythrina* memberikan efek anti kanker pada sel kanker payudara T47D, anti plasmodium in vitro terhadap strain K1 dan 3D7 strain parasit, dan anti kesuburan pada spermatozoa *R. norvegicus* aktivitas sedang dan tinggi. Selain itu, uji toksisitas dilakukan pada hewan coba tikus putih, dan hasilnya praktis tidak toksik. **Simpulan** Hasil penelitian ini menunjukkan bahwa *E. variegata* efektif untuk digunakan sebagai obat herbal untuk anti kanker, anti malaria, dan agen anti kesuburan.

Kata kunci : anti kanker, anti kesuburan, antimalaria, *Erythrina variegata*.

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INTRODUCTION

Indonesia is one of the richest countries in plant resources. In particular, the variety of plant species is enormous, so Indonesian medicinal plants are very important source of new natural bioactive compounds. 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 behavioral factors are more important than genetic factors in determining overall cancer frequency among populations. Currently, systemic cytotoxic chemotherapy

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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

Malaria is one of the most important health problem in Indonesia. Medicinal plants are commonly used particularly in the rural regions to treat malaria and associated symptoms. Recently, the acceptance of traditional medicine as an alternative form of health care has increased among all socio-economic groups of the population, and phytomedicine has become an important economic sector 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 The search for anticancer, antifertility, and antimalarial medicinal plant depends on the accurate and specific ethnobotanical and ethnopharmacological information obtained from the reference document. Recently, attention was focused on medicinal plants to provide new anticancer, antifertility, and antimalarial agents. *Erythrina variegata* (Leguminosae) is a famous medicinal plant widely distributed in tropical and subtropical region of the world. This plant is locally known as “dadap ayam” in Indonesia and the leaves of *E. variegata* are used as an antimalarial agents^{3,4,5,6,7,8,9,10} Pharmacological report indicated that a diterpenoid derivates, phytol from the leaves of *E. variegata* showed antifertility activity.¹¹ The flavonoid and steroids derivates isolated from the leaves of *E. variegata* show anticancer activity against breast cancer cell T47D.^{8,10} As part of our continuing search for novel anticancer, antifertility, and antimalarial from Indonesian *Erythrina* plants, we report here with the isolation, structure elucidation and anticancer, antifertility, antimalarial activity from the leaves of *E. variegata*.

EXPERIMENTAL SECTION

General Experimental Procedure.

Melting point was determined on Fisher Johns apparatus. The UV and IR spectra were obtained on Varian 100 and Perkin Elmer spectrophotometers, respectively. NMR spectra was recorded with a JEOL JNM A-400 spectrophotometer using TMS as an internal standard. Vacuum liquid chromatography was carried out using Merk silica gel 60 GF₂₅₄ (230-400 mesh) and open column liquid chromatography using Merk silica gel 60 GF₂₅₄ (70-230 mesh), and TLC analysis on precoated Kieselgel 60 GF₂₅₄ 0.25 mm. The analysis of spermatozoa was determined using hemositometer improved Neubauer and microscope.

Plant Material.

Samples of the leaves of *E. variegata* were collected in Desember 2008, from Subang Distric, West Java, Indonesia. The plant was identified at the Herbarium Bandungensis, Department of Biology, Insitut Teknologi Bandung, Indonesia, and voucher specimen had been deposited at the herbarium.

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Animal Material.

Adult male rats (*R. norvegicus*) were obtained from the Structural and Development of Animal Laboratory Department of Biology, Faculty of Mathematics and Natural Sciences, Padjadjaran University, Jatinangor, Sumedang, Indonesia.

Parasite strain

In this study two strains of *P. falciparum* were used, culture of 3D7 (chloroquine sensitive) and K1 (chloroquine resistant).

Extraction and isolation

The dried leaves (2 kg) of *E. variegata* were soaked in methanol. Evaporation of the methanol gave an aqueous concentrate, which was extracted with methylenedichloro. The resulting methylenedichloro extract was partitioned between n-hexane and methanol containing 10% water, and then the lower layer was concentrated and extracted with ethyl acetate to afford residue (36.31 g). The methanol layer was partitioned between n-butanol-water (3:1). The ethyl acetate layer was subsequently dried over anhydrous sodium sulfate, filtered, evaporated to dryness, and assayed for anti-malarial activity. The ethyl acetate fraction (10.5 g) was chromatographed on Kieselgel 60 (70-230 mesh) by eluting with chloroform-ethyl acetate gradient (1:1-1:5) to yield a 10 fractions (FA-J). The FJ fraction (635 mg) was eluted with chloroform and 5% acetic acid were further flash-chromatographed on Kieselgel 60 to yield a compound **1** (24.5 mg). The n-butanol layer was subsequently dried over anhydrous sodium sulfate, filtered, evaporated to dryness, and assayed for anti-malarial activity. The n-butanol fraction (4 g) was chromatographed on Kieselgel 60 (70-230 mesh) by eluting with chloroform-ethyl acetate in an increasing ratio (1:1-1:5) to yield a 3 fractions (BA, BB, and BC). The BC fraction (1.5 g) was eluted with chloroform and 5% acetic acid were further flash-chromatographed on Kieselgel 60 to yield a compound **2** (53 mg).

Anticancer Assay

National Cancer Institute developed an *in vitro* anticancer-drug method with the SRB (Sulforhodamine B) assay. This method measures the cellular protein content of adherent and suspension cultures in 96-well microtiter plates. Cultures fixed with trichloro acetic acid (TCA) are stained with 0.4% SRB dissolved in 1% acetic acid. Unbounded dye is removed by washing with 1% acetic acid, and protein-bound dye is extracted with 10 mM buffered tris base [tris (hydroxymethyl) amino methane] for the determination of optical density (515 nm) with a 96-well microtiter plate reader. Cisplatin functioned well as positive controls.¹²

Antimalarial assay

In vitro testing of the antimalarial activity was carried by measuring the LDH activity of the parasite. Briefly, continuous culture of the 3D7 sensitive chloroquine and K1 resistant chloroquine, were

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maintained in a suspension consisting of RPMI 1640 culture medium supplemented with HEPES, N-2-hydroxyethylpiperazine-N'-2-ethane-sulfonic acid (25 mM), sodium bicarbonate (0.2%) and gentamycin (40 µg/mL) at pH 7.4, and O type red blood cell. For each LDH test, a blood suspension of 1% parasitemia and 2% haematocrit were prepared. Control reading of parasitized red blood cells devoid of plant extracts or drugs and non-parasitized red blood cells were done simultaneously. After the plate had been prepared, it was placed in a candle jar and incubated for 48 h at 37°C. After 48 h, 100 µl of Malstat (Flow Inc., Portland, OR), was dispensed into a new microtitreplate. An amount of 25 µl of NBT-PES (Sigma Chemicals, USA) mixture was then added. Twenty microliters of blood suspension was transferred into the plate containing the Malstat and NBT-PES. Any air bubbles were eliminated as it could interfere with the absorbance reading. Absorbance was read at 630 nm using an ELISA plate reader (MRX Microplate Reader, Dynex Technologies, USA). Chloroquine and artemisinin functioned well as positive controls.¹³

Antifertility assay

Samples were tested at 0.25 µg/µL on spermatozoa of white rat *in vitro*. Spermatozoa were collected from cauda epididymides. The spermatozoa suspension was prepared in 10 drops of 0.9 % saline, and then diluted 1000 x in 0.9 % saline. A 25µL of spermatozoa suspension was mixed with sample and the motility, viability, and abnormality were analysed using hemocytometer improved Neubauer and microscope. The control was analysed by the same method without sample.¹⁴

RESULTS AND DISCUSSION

The methanol extract of dried leaves of *E. variegata* exhibited an antimalarial activity *in vitro* against *Plasmodium falciparum*, anticancer activity *in vitro* against breast cancer cell T47D, anti-fertility on spermatozoa of white rat (*R. norvegicus*) *in vitro*. The active methanol extract was partitioned between n-hexane, ethyl acetate and n-butanol to afford a n-butanol fraction. By using the bioassay to follow the separations, ethyl acetate and n-butanol fractions were separated by combination of column chromatography on Kieselgel 60 to afford an active compounds (**1** and **2**).

The compound **1** was obtained as a white solid and can be decomposed at a temperature of 241-243°C. The compound **1** showed the molecular formula C₃₆H₅₈O₆ based on ¹H- and ¹³C-NMR. The HMBC spectrum showed that H-6 (δ_H 5.08 ppm) and H-7 (δ_H 5.21 ppm) correlated with C-5 (δ_H 51.8 ppm) and C-9 (δ_H 41.2 ppm), the correlation also occurs between H-6 (δ_H 5.08 ppm) with C-7 (δ_H 139.2 ppm), and opposite there is a correlation between H-7 with C-6, the correlation between H-11 (δ_H 1.55 ppm) with C-12 (δ_H 122.3 ppm) and C-13 (δ_H 139.2 ppm). The correlation between H-2 (δ_H 4.98 ppm) and H-1' (δ_H 2.49 and 2.77 ppm) with C-3 (δ_H 79.0 ppm). Protons at position 1' and 2' has its value 3J = 7.95 Hz certain so that protons 1' and 2' axial-axial position. This suggests that compound **1** contains residual sugar that position β¹⁵, and compound **1** as terpenoid pentacyclic glycoside (Fig.1).

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The compound **2** was obtained as a yellow pale needless crystal, m. p. 72-74 °C. The molecule formula was established to be C₁₉H₂₁NO₆, m/z 359 by EIMS spectral data, thus requiring ten degrees of unsaturation. Its UV spectrum of isolate showed aryl and carbonyl absorption at 288 and 350 nm, respectively. The IR spectrum of compound isolate displayed some characteristic absorption for an aromatic ring, hydroxyl and carbonyl group. The ¹H-NMR and ¹³C-NMR spectra of isolate showed signals assignable to a 1,2,4,5-tetrasubstituted benzene ring [δ_{H} 7.02 (1H,s) and 7.47 (1H,s)] and [δ_{C} 107.8; 110.4; 124.3; 139.2; 149.3; and 153.1] and two carbonyl groups [δ_{C} 160.0 and 180.4] ppm, indicating isolate to be a tetracyclic structure. Three methoxyls were also observed in the ¹H-NMR and ¹³C-NMR spectra [δ_{H} 3.16 (3H, s); 3.95 (3H, s) and 3.98 (3H, s)] and [δ_{C} 57.2; 56.3 and 56.5]. To determine the connectivity of the partial structure, ¹H-¹H COSY, HMBC, and NOESY experiment for isolate was carried out, and the results are shown in Figure 1. The NOESY spectra showed signals assignable to α -configured equatorial of H-1, H-2, H-3, H-4, H-7, and H-8, while of 2-OH, 3-OCH₃, H-4, H-7, H-8, H-14, 15-OCH₃, 16-OCH₃, and H-17 are β -configured axial. The based on the spectral spectroscopic evidence, comparison with the previously reported and biogenetic point of view, the genus *Erythrina* seems to lack biogenetic ability to produce alkaloids [16,17] compound **2** identified as 10,11-dioxoerythratidine (Fig.1).

The potency of methanol extract, ethyl acetate fraction, terpenoid pentacyclic glycoside, and 10,11-dioxoerythratidine against breast cancer cell T47D can be described to be in the following order; 10,11-dioxoerythratidine > terpenoid pentacyclic glycoside > ethyl acetate fraction > methanol extract, indicated that 10,11-dioxoerythratidine and terpenoid pentacyclic glycoside to be potential as anticancer agents (Table 1).

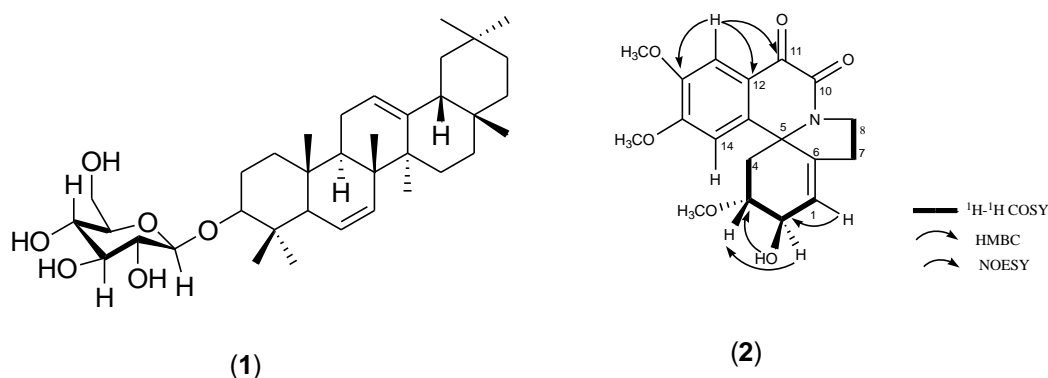


Fig.1 Structure (1) and Correlation ¹H-¹H COSY, HMBC, and NOESY (2)

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Table 1. IC₅₀ values of methanol extract, ethyl acetate fraction, n-butanol fraction, terpenoid pentacyclic glycoside, and 10,11-dioxoerythratidine against breast cancer T47D cell-line

Sample	IC ₅₀ (µg/ml)
Methanol extract	43.7
Ethyl acetate fraction	22.9
n- butanol fraction	10.5
terpenoid pentacyclic glycoside	3.2
10,11-dioxoerythratidine	1.0
Cisplatin	3.3

Inhibition of the methanol extract towards resistant strain (K1) was also better compared to its inhibition towards the sensitive strain (3D7) of the parasite (Table 2). The methanol extract (IC₅₀ 6.8 µg/mL) showed strong antiplasmodial activity against K1 strain of the parasite based on the threshold for in vitro antiplasmodial activity of antimalarial extracts.¹⁵ The potency of methanol extract, indicated that the leaves of *E. variegata* were potential candidate for antimalarial agent. The leaves of *E. variegata* was widely used in traditional medicine³ and it was boiled in water and used for malaria treatment.⁴ The ethyl acetate fraction showed weak antiplasmodial activity against both strains of the parasite with IC₅₀ of 26.5 µg/mL and 16.7 µg/mL against K1 and 3D7 respectively. Previously, antimalarial triterpenoid pentacyclic isolated from ethyl acetate fraction of the leaves of *E. variegata* toward *P. falciparum* FCR-3/A (chloroquine resistant).⁹ The n-butanol fraction (IC₅₀ 5.1 µg/mL) showed good antiplasmodial activity against K1 strain of the parasite, but it showed moderate antiplasmodial activity against the 3D7 strain (IC₅₀ 13.2 µg/mL). The terpenoid pentacyclic glycoside (IC₅₀ 3.3 µg/mL) from ethyl acetate fraction showed good anti-plasmodial activity against K1 strain of the parasite, but it showed weak anti-plasmodial activity against the 3D7 strain (IC₅₀ 1.8 µg/mL). The 10,11-dioxoerythratidine (IC₅₀ 3.2 µg/mL) from n-butanol fraction showed strong anti-plasmodial

Table 2. The IC₅₀ of methanol extract, ethylacetate fraction, n-butanol fraction, terpenoid pentacyclic glycoside, and 10,11-dioxoerythratidine against *P. falciparum* K1 and 3D7 strains

Sample	IC ₅₀ (µg/mL)	
	K1	3D7
Methanol extract	6.8	>60
Ethyl acetate fraction	26.5	16.7
n- butanol fraction	5.1	13.2
terpenoid pentacyclic glycoside	3.3	1.8
10,11-dioxoerythratidine	3.2	9.3
Chloroquine	0.04	0.04
Artemisinin	0.01	0.01

activity against K1 strain of the parasite, but it showed weak anti-plasmodial activity against the 3D7 strain (IC₅₀ 9.3 µg/mL). Result from this in vitro antimalarial study showed that the activity of ethyl

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acetate fraction (IC₅₀ 26.5 µg/mL), n-butanol fraction (IC₅₀ 5.1 µg/mL), compound **1** (IC₅₀ 3.0 µg/mL) and compound **2** (IC₅₀ 3.2 µg/mL) against chloroquine resistant *P. falciparum* K1 strain can be described in the following order; terpenoid pentacyclic glycoside >10,11-dioxoerythratidine > n-butanol fraction > ethyl acetate fraction. The terpenoid pentacyclic glycoside and 10,11-dioxoerythratidine were the most potent fraction towards the target *P. falciparum* K1 strain compared to those of n-butanol fraction, methanol extract, and ethyl acetate fraction.

The result of antifertility testing on methanol extract, ethyl acetate fraction, 10,11-dioxoerythratidine showed anti-fertility activity were listed in Table 3. Compared to the control, the quality of spermatozoa was significantly decreased in both motility and viability, but increase in abnormality, after treating with the samples (Table 3).

Table 3. The motility, viability, and abnormality of *R. novergicus* spermatozoa after treated with methanol extract, ethyl acetate fraction, n-butanol fraction, and 10,11-dioxoerythratidine

Sample	Reducing of motility (%)	Reducing of viability (%)	Increasing of abnormality (%)
Methanol extract	60.90	70.47	80.17
Ethylacetate fraction	59.76	65.99	62.13
n-butanol fraction	70.55	85.14	70.20
10,11-dioxoerythratidine	80.41	95.98	82.99

It might be an indication that a terpenoid pentacyclic glycoside and 10,11-dioxoerythratidine were potential candidate for anticancer, antifertility, and antimalarial agents. The results obtained from this study also indicated that a variety of secondary plant metabolites displayed anticancer activity against breast cancer cell T47D, antimalarial activity against *P. falciparum*, and antifertility activity on spermatozoa of white rat (*R. novergicus*) in vitro. The terpenoid pentacyclic glycoside and 10,11-dioxoerythratidine is the first report on anticancer, antimalarial, and antifertility agents.

CONCLUSIONS

The terpenoid pentacyclic glycoside (**1**) and 10,11-dioxoerythratidine (**2**) had been isolated from the leaves of *E. variegata*. Our results strongly suggested that the leaves of *E. variegata* is promising as herbal medicine anticancer, antimalarial, and antifertility agents.

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