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Research Article

IN VITRO ANTIMALARIAL ACTIVITY OF CHLOROFORM, N-BUTANOL, AND ETHYL ACETATE FRACTIONS OF ETHANOL EXTRACTS OF *CARTHAMUS TINCTORIUS* LINN. FLOWERS

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ABSTRACT

Objective: This objective of this research was to study *in vitro* antimalarial activity of chloroform, n-butanol, and ethyl acetate fractions of ethanol extracts of *Carthamus tinctorius* Linn. flowers from Asteraceae family which empirically been used as traditional medication by people in South Sulawesi to heal measles.

Methods: Fractionation was conducted using chloroform, n-butanol, and ethyl acetate. Determination of antimalarial activity was performed by *in vitro* test using the 24-well microplate and the candle-jar method. Breeding is done in a petri-dish and done aseptically. *Plasmodium falciparum* 3D7 culture obtained from frozen deposits in-thawing and bread from Pharmacy Laboratory of Airlangga University, Surabaya, Indonesia. Blood sample with a density of over 2000 was employed. Serial decreasing concentrations of the crude extract of chloroform, butanol, and ethyl acetate fraction were tested for antimalarial activity. The following concentrations were used; 100; 10; 1.0; 0.1; and 0.01 (mg/mL). Negative controls used dimethyl sulfoxide (DMSO) diluted in the same manner as diluting materials above test, to obtain final DMSO concentration is not more than 0.5%. Mixture and suspension test parasites (= test preparation) are then inserted into the candle-jar and incubated in a CO2 incubator at a temperature of 37°C for 48 h. After incubation for 48 h made a thin blood smear on glass object. Smear dried at room temperature, fixed with methanol, then, once dry stained with Giemsa and counted under a microscope parasitemianya with 1000 times magnification. Calculations performed on 5000's erythrocytes.

Results: Results showed that chloroform and n-butanol fraction cannot inhibit parasitemia >50%, but ethyl acetate fraction can inhibit parasitemia >50% with the highest inhibition at 100 µg/mL of 94.48%.

Conclusion: Ethyl acetate fraction is highly active as antimalarial with an IC_{50} of 1.25 µg/mL.

Keywords: Antimalarial activity, *In vitro*, *Plasmodium falciparum* 3D7, *Carthamus tinctorius* Linn.

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INTRODUCTION

Malaria is an infectious disease caused by protozoa of the genus *Plasmodium*. This disease contributes to the high rate of mortality among infants, under-five children, and pregnant women. Annually, more than 500 people worldwide are infected by malaria and 1 million died due to this disease. The majority of malarial cases occur in Africa, as well as in several countries in Asia, South America, Middle East, and Europe. To deal with the disease, the 60th meeting of the World Health Assembly has come up with a global commitment on the elimination of malaria in all countries. Guidance on how to go about doing this has been provided by the World Health Organization through its Global Malaria Program [1].

Indonesia is one of the countries at the risk of malaria. Most of Indonesian regions are malaria-endemic, in particular, the eastern part of the country including Papua, Maluku, Nusa Tenggara, Sulawesi, and Kalimantan, as well as some areas in Lampung, Bengkulu, and Riau. In some areas of Java and Bali, despite low endemism, cases of malaria outbreaks still occur frequently. In 2007, 396 out of 495 regencies in Indonesia are malaria-endemic, and it was estimated that 45% of the population live in areas that are at high risk of malaria infection. In addition, malaria morbidity in the country is reported to rise from year to year. To overcome malaria disease, various efforts have been made both in global and national scale. Malaria is one of the indicators of Millennium Development Goals, with an aim at putting a stop to the spread of malaria and reducing malaria-related incidents in 2015, to be indicated by a decrease in morbidity and mortality caused by malaria [2].

One factor, leading to some problems faced in the eradication of malaria, is a decrease in the efficacy of antimalarial drugs that have commonly been taken. *Plasmodium falciparum* has been reported to be resistant to antimalarial chloroquine. Reports also indicate that resistance to chloroquine, sulfadoxine-pyrimethamine, mefloquine, halofantrine, and quinine occur in some areas in Southeast Asia. In certain areas within the region, a combination of artemisinin and its derivatives is the only effective medication [3]. Other factors leading to the still high rate of morbidity and mortality include large-scale human migration, environmental and climate change, poor health-care system, and the emergence of strains of insecticide-resistant Anopheles mosquitos [4]. However, this has drawn the attention of the researcher and active research is going on for the exploration of new sources of lead molecules that can be eventually developed into potential alternative antimalarial drugs [5].

An alternative way of preventing malaria is by developing a concept of immunotherapy medication, that is, an administration of drugs in the form of either synthetic or natural compounds (plants) that are capable of optimizing the functions of all components in human body immune system to combat malarial infection. Immunity to malarial infection involves cellular and humoral responses. A cellular immune response is mediated by T-lymphocytes, which plays an important role in the fight against the infection from intracellular sporozoites (skizogoni extraerythrocytic).

Kasumba turate (*Carthamus tinctorius* Linn.) flower from the Asteraceae family has empirically been used as traditional medication

by people in South Sulawesi to heal measles by brewing the plant in hot water before taking its juice, the consumption of which is believed to help increase body's immunity system. A study on the ethanol extract of *C. tinctorius* Linn. discovered that the extract highly significantly increases both immunoglobulin G (IgG) and A (IgA) activities. Dried flowers of this plant can also be used as emmenagogue, laxant, or stimulant. In addition to consumption, its seed oil is used in mixture of cosmetic materials [6]. When tested on mice to determine its cellular immune response, the immunomodulatory effect of juice extracted from Kasumba turate flower (*C. tinctorius* Linn.) was remarkably significant. A test on non-specific immune response showed that a dosage of 1.95 and 3.90 mg/kg bodyweight could increase phagocytosis speed as was indicated by the phagocytosis index: 1.72 and 1.88 (K > 1.5), and this was considered as a strong immunostimulation. This increases very significantly when the flower is brewed with hot water [7]. Ethanol extract of *C. tinctorius* Linn. showed a potential antimalarial activity with an IC_{50} 1.06 µg/mL. Therefore, the present study is designed to evaluated antimalarial activities of various fractions (chloroform, N-butanol, and ethyl acetate fraction) of ethanol *C. tinctorius* flowers extract.

However, many malaria-affected areas of Assam where people entirely rely on ethnomedicinal practices are not yet explored. In the present study, an ethnobotanical survey was conducted in highly malaria-affected seven districts, namely, Goalpara, Baksa, Kamrup, Morigaon, Nagaon, Dibrugarh, and Dhemaji of Assam and all the gathered information were scientifically analyzed and validated.

METHODS

Plant material

Flowers of Kasumba turate (*C. tinctorius* Linn.) were collected from bone, a regency in South Sulawesi and have previously been determined by Purwodadi LIPI in Pasuruan.

Extraction

3 kg of *C. tinctorius* Linn. flowers was extracted with 10 L of ethanol. The ethanolic extract was concentrated to dryness on rotatory evaporator. 178.7 g of crude extract was suspended in distilled water and partitioned with chloroform, butanol, and ethyl acetate successively, which were in turn concentrated to dryness in vacuo on rotary evaporator. The ethanolic extract of the fraction was tested for it's *in vitro* antimalarial activities on *P. falciparum* 3D7 using the 24-well µL plate and the candle-jar method. Breeding is done in a petri-dish and done aseptically. *P. falciparum* 3D7 culture obtained from frozen deposits in-thawing and bread from Pharmacy Laboratory of Airlangga University, Surabaya, Indonesia. Blood sample with a density of over 2000 was employed. Serial decreasing concentrations of the crude extract of chloroform, butanol, and ethyl acetate fraction were tested for antimalarial activity. The following concentrations were used; 100; 10; 1.0; 0.1; and 0.01 (mg/mL). Negative controls used dimethyl sulfoxide (DMSO) diluted in the same manner as diluting materials above test, to obtain final DMSO concentration is not more than 0.5%. Mixture and suspension test parasites (= test preparation) are then inserted into the candle-jar and incubated in a CO_2 incubator at a temperature of 37°C for 48 h. After incubation for 48 h made a thin blood smear on glass object. Smear dried at room temperature, fixed with methanol, then, once dry stained with Giemsa and counted under a microscope parasitemianya with 1000 times magnification. Calculations performed on 5000's erythrocytes.

Data analysis

The data obtained from *in vitro* antimalarial activities assay above is in the form of a number of parasite-infected erythrocytes (counted on around 5000 erythrocytes) were subsequently converted into parasitemia levels (percent parasitemia) and the percent inhibition of the test substance on the growth of the parasite.

Table 1: Mean percentage inhibition and IC_{50} each partition against *P. falciparum* 3D7

Fraction	Concentration (µg/mL)	Percent inhibition (%)	IC_{50} (µg/mL)
Chloroform	100	20.16	-
	10	6.36	
	1	0	
	0.1	0	
	0.01	0	
	K(-)	-	
N-Butanol	100	37.21	5007,432
	10	26.56	
	1	19.97	
	0.1	19.11	
	0.01	10.12	
	K(-)	-	
Ethyl acetate	100	94.48	1.252
	10	63.19	
	1	44.85	
	0.1	26.50	
	0.01	10.99	
	K(-)	-	

P. falciparum: Plasmodium falciparum

Percent parasitemia calculated by the formula:

$$\% \text{ Parasitemia} = \frac{\sum \text{infected erythrocytes}}{\text{The number of erythrocytes}}$$

The percentage inhibition of parasite growth is calculated using the formula:

$$\% \text{ Inhibition } = 100\% - \left[\frac{Xp}{Xk}\right] \times 100\%$$

Based on the data about the inhibition and concentration of the tested materials, IC_{50}, or the concentration of test materials that inhibit parasite growth by 50%, was then calculated using the probity analysis.

RESULTS

The crude ethanolic extract of *C. tinctorius* flowers inhibited *P. falciparum* 3D7 with IC50 = 1.06 µg/mL. *In vitro* antimalarial assay of *P. falciparum* strain 3D7 was conducted on the chloroform, butanol, and ethyl acetate fraction. The detailed results for each of partition are as shown in Table 1.

DISCUSSION AND CONCLUSION

The present study confirms the antimalarial activity fraction chloroform, butanol, and ethyl acetate of *Carthamus tinctorius* Linn. flowers. Chloroform fraction is not exhibited an IC_{50}, so this fraction did not have antimalarial activities. Butanol fraction exhibited an IC_{50} 5007,43 µg/mL, and ethyl acetate fraction can inhibit parasitemia >50% with the highest inhibition at 100 µg/mL of 94.48%. Ethyl acetate fraction is highly active as antimalarial with an IC_{50} of 1.25 µg/mL. According to Kohler (2002), an extract whose IC_{50} value is lower than 50 (µg/mL) and a fraction whose IC_{50} value is lower than 25 (µg/mL) is considered effective antimalarial. Data on Table 1 summarize that the IC_{50} values of ethyl acetate fraction are lower than Kohler's criteria. It can, therefore, be stated that the ethyl acetate fraction can serve as effective antimalarial. However, since the IC_{50} value of ethanolic extract is lower than ethyl acetate fraction, it can be concluded that ethanol has a more potential antimalarial activity. Therefore, it is necessary to isolate of *C. tinctorius* Linn. flowers according to the bioassay-guided isolation

principle to obtain active compounds that can be used as a marker in the standardization of antimalarial materials, so that *C. tinctorius* Linn. flowers can be developed into phytopharmaca products.

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