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In Vitro Antiplasmodial Activity of Ethanol Extract of Manuran (*Coptosapelta Tomentosa* Valeton Ex K. Heyne) Roots.

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ABSTRACT

The efforts to find alternative antimalarial medicine have not essentially solved the problem of malaria control because of the presence of medication resistance. The *Plasmodium* resistance to antimalarial medicine is resulted from malaria treatment failure. Therefore, the availability of new antimalarial medicine is urgently needed to overcome the resistance problems. *Coptosapelta tomentosa* is empirically used by people in Kotabaru South Kalimantan as part of the treatment for Malaria. The aim of this research was to determine IC_{50} value of *in vitro* antiplasmodial activities from ethanol extract of *C. tomentosa* roots. Coarse powder of *C. tomentosa* roots was extracted using ethanol 96% by maceration method. An *in vitro* antiplasmodial activity test was carried out by using Candle Jar method. The doses of administration of ethanolic extract *of C. tomentosa roots were at* 500; 250; 50; 25; 5; dan 0.5 µg/mL concentration. The results of the study included parasitemia percentage, *P. falciparum* growth inhibition percentage and IC_{50} value. The IC_{50} value of the ethanol extract of *C. tomentosa* roots was 2.46 µg/mL. The *in vitro* antiplasmodial activities of ethanol extracts of *C. tomentosa* roots were categorized to be very active.

Keywords: *Captosapelta tomentosa*; Manuran; *in vitro* antiplasmodial activity; Borneo; Kalimantan

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INTRODUCTION

The resistance of *Plasmodium* to antimalarial drugs resulted in treatment failure. It posed a threat in the absence of alternative effective drug for the resistance [1]. Therefore, the availability of new antimalarial drug was highly required to overcome this problem. Exploration of new antimalarial drugs was conducted using various methods, including exploration and development of natural substance. The selection of the natural substance was usually conducted on the basis of empirical use by people. Exploration was conducted by Prozesky et al [2] to 14 species of plants traditionally used as an antimalarial drug by communities in South Africa. The results showed that more than 50% of the species inhibit the proliferation of malaria parasites at 50 µg/mL. The strongest antiplasmodial activityis in the extracts of dichloro methane for *Ozoroaengleri* and *Balanites maughamii* with the same inhibition(IC_{50}1.7µg/mL).

A study conducted by Arnida et al [3] is to test *in vitro* antiplasmodial activity against the ethanolic extracts of *Hydrolea spinosa* leaves*; Ampelocissus rubiginosa* tubers*; Uraria crinite* roots*; and Angiopteris evecta* tubers. The results of the study showed that *A. evecta* tubers potentially (IC_{50} value of 2.858±0.27 µg/mL) have *in vitro* antiplasmodial activity. Manuran (*Coptosapelta tomentosa)* roots was used by people living in Kotabaru of South Kalimantan to treat malaria. Though the plants have been empiricallyused to treat the malaria, there has not been any publication of the study of *in vitro* antiplasmodial activity. Therefore, the recent study gave to find out *in vitro* antiplasmodial activity of the active compound of the *C. tomentosa* roots in *P. falciparum strain* FCR3 culture. The *in vitro* antiplasmodial activity were categorized on the basis of the resulting IC_{50} values.

MATERIALS AND METHODS

Plant Materials:

The parts of the plants used in the study were the roots of the *C. tomentosa,* that were collected from Kotabaru of South Kalimantan.

Experimental animals:

The used parasites were *P. falciparum strain* FCR3 obtained from the Pharmacology Laboratory of the Faculty of Medicine of Universitas Gadjah Mada Yogyakarta.

Chemical Material

The materials used for the fractionation were n-hexane, ethyl acetate, ethanol, methanol. RPMI, HEPES, $NaHCO_3$, gentamicin, RBC (Red Blood Cell) of blood group O, sodium chloride 0.9%; 1.6%; 12%, human blood serum with blood group O, wax, 10% glycerol, 5% sorbitol, methanol, DMSO, chloroquine, distilled water, alcohol, 0.2% dextrose, glycerol, freezing medium, giemsa, and oil immersion were used to test *in vitro* antiplasmodial activity.

Methods:

Extraction

The collected parts of the plants were washed using tap water, chopped into small pieces and dried in oven at 50^{o}C. Dried powder of the *C. tomentosa* roots*,* were macerated using ethanol 96% for 24 hours. The resulting filtrate was evaporated to obtain thick ethanol extract.

Phitochemistry Screening

The Phitochemistry screening of ethanol extracts of *C. tomentosa* roots were alkaloids, flavonoids, steroids-terpenoids, anthraquinone, saphonins, and tannins tests.

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***In Vitro* Antiplasmodial Activity Test**

The antiplasmodial activity test was carried out to the ethanol extract of *C. tomentosa* roots. It was carried out using candle jar method [4]. The culture was maintained by replacing the media every 24 hours. If the parasitemia was too high (i.e., more than 10%), subculture was prepared by adding red blood cells so that it become lower. If the *Plasmodium* culture has grown and reached more than 2%, the test might be carried out by doing synchronization. The material was weighted, added 100 µL DMSO and 900 µL RPMI solutions and sterilized by filtering it using 0.20 µm membrane filter. Concentration ranks of the materials were then made, viz. 500, 250, 50, 25, 10, and 0.5 µg/mL. Chloroquine was used as positive control and its concentration ranks were made, viz. 40, 20, 16, 12, 8 and 4 µg/mL. The testing material, negative control (i.e., RPMI media) and 100 µL chloroquine were put into microplate (with 96 wells) and added 100 µL of *Plasmodium* resulting from the synchronization. The microplate was put into the candle jar, incubated at 37 ^{o}C for 72 hours. Once the incubation period has completed, the microplate was take out of the candle jar and harvesting was done by moving the mixture of each of the wells into microtube. Subsequently, it was centrifuged and its supernatant was removed. The residue was used to prepare smears on glass object. Once the smears have dried, they were fixed using methanol. The dried smears were painted using 5% Giemsa paint, left idly for 30 minutes and washed using tap water. They were aerated till completely dry and then added emersion oil. The number of erythrocytes and parasitemia of the smears might be clearly observed and calculated under microscope. The data of the parasitemia percentage of each test was compared to that of the negative control that parasite growth inhibition percentage (%) was obtained, with the formula:

$$\%Inhibition = \frac{ParasitaemiaNegativeControl - ParasitaemiaTest}{\sum Erythrocites} x100\%$$

Data Analysis:

The resulting data was presented in the form of correlation curve between the testing compound concentration and the parasite growth inhibition percentage. The IC_{50} was determined using probit analysis of the inhibition percentage with testing concentration logarithm. The resulting IC_{50} data was classified on the criteria that the extract of the medicinial plants were considered to have no antiplasmodial activity if the IC_{50} value was more than 50 µg/mL [5].

RESULTS AND DISCUSSION

Manuran were identified by comparison with authentic specimens at the Herbarium Bogoriense, Research Center for Biology, Indonesian Institute of Science Indonesia (LIPI). A voucher specimen is kept in Program Study of Pharmacy, Faculty of Mathematics and Natural Sciences, Universitas Lambung Mangkurat South Kalimantan, Indonesia with number 297/IPH.1.01/If.07/II/2016.

The ethical clearance was gained at Medical and Health Research Ethics Committee (MHREC) Faculty of Medicine, Universitas Gadjah Mada, Yogyakarta, the approval Ref. KE/FK/109/EC states that the above protocol meets the ethical principle outlined in the declaration of Helsinki 2008.

The drying decrease of 21.1% indicated that the water content of the *C. tomentosa* roots was not dominant. Simplisia had dense and hard texture, and it was difficult to process it into fine powder so that in most cases the resulting powder was coarse. One and a half kilogram of the simplisia was extracted using ethanol and gave 229.25 g dry extract, meaning that the randement of the ethanol extract was 15.28 % w/v. Based on the results of the phytochemical screening, the ethanol extract of *C. tomentosa* roots contained flavonoids, tannins, triterpenoids, saponins, and anthraquinone (Table 1).

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Table 1. The results of the phytochemical screening, the ethanol extract of *C. tomentosa* roots

Num.	Identification	Observations	Concl.
1.	Alkaloids		
	Reagent Dragendorff	no precipitate	(-)
	Reagent Mayer	no precipitate	(-)
2.	Flavonoids		
	Reagent ammonia vapor	filter paper on orange	(+)
3.	Tannins		
	Reagent gelatin 1%	white precipitate	(+)
	Reagent $FeCl_3$	black	(+)
4.	Steroids and Triterpenoid		
	Reagent Liebermann-Burchard	ring of brownish	(+)
5.	Saponins		
	Foam test	forming foam	(+)
6.	Anthraquinone		
	Reagent Borntraeger	aliquot red	(+)

***In Vitro* Antiplasmodial Activity**

The results of the *in vitro* antiplasmodial activity test of ethanol extracts of *C. tomentosa* roots in *triplet* were mean parasitemia percentage, inhibition, and IC_{50} value (Table 2). The parasitemia percentage resulted from the percentage of the infected erythrocyte number per thousand erythrocytes. The parasitemia percentage was increasingly smaller with the increase in the concentration of the fractions in the test. The calculation of the parasitemia percentage and negative control gave the percentage of *Plasmodium* growth inhibition. The bigger was the concentrations of ethanol extracts of *C. tomentosa* roots, the stronger the inhibition of the *Plasmodium* growth by the chloroquine was. The antiplasmodial activities of the ethanol extracts of *C. tomentosa* roots were manifested in the inhibition concentration of 50% (IC_{50}) calculated using probit analysis of SPSS 16 program.

Table 2. The Percentages of paracitemia, Plasmodial inhibitory, and IC_{50} Value

Sample	Concentration (µg/mL)	%Paracitemia	%Plasmodial Inhibition	IC_{50} Value (µg/mL)
Ethanol Extract *C.tomentosa*	500	1.45	90.38	2.457
	250	1.80	88.01	
	50	3.73	75.15	
	25	4.48	70.15	
	5	5.26	64.96	
	0.5	10.39	30.79	
Chloroquine (Arnida et al, 2015)	$40x10^{-3}$	0.00	100	$4.807x10^{-3}$
	$20x10^{-3}$	0.044	99.45	
	$16x10^{-3}$	0.161	98.02	
	$8x10^{-3}$	0.205	97.48	
	$6\ x10^{-3}$	0.304	96.26	
	$4x10^{-3}$	5.964	26.61	

The *in vitro* antiplasmodial activities of ethanol extracts of *C. tomentosa* roots were categorized on the basis of the resulting IC_{50} values was 2.457 µg/mL. The *in vitro* antiplasmodial activity was considered to be inactive if IC_{50} was more than 100 µg/mL [6], less active if the IC_{50} value was around 50 – 100 µg/mL, active if the IC_{50} value was around 5-50 µg/mL [6; 7], and very active or very potential if it has IC_{50} was less than 5 µg/mL [6; 7; 8; 9]. The *in vitro* antiplasmodial activities of the ethanol extracts of *C. tomentosa* roots were as follows was categorized to be very active. If the IC_{50} values of the chloriquine (IC_{50} $4.807x10^{-3}$ µg/mL) and the

ethanol extract of the *C. tomentosa* roots (IC_{50} 2.457 µg/mL) were compared, they were very different, but their *in vitro* antiplasmodial activity was categorized to be the same, which was very active. However, they might have significant difference because of the difference in the working mechanism of the medicine and other influencing factors that must be scientifically proven in other studies.

The identification of terpenoid in the ethanol extract of the roots of the *C. tomentosa* was positive, but the result of steroid test was negative. Terpenoid or steroid compound would dehydrate with the addition of strong acid and formed salt that caused a number of color reactions. The test of the steroid and the triterpenoid was based on the capability of the compounds to produce color with concentrated H_2SO_4 in anhydrous acetic acid. The examination of the triterpenoid and the steroid was conducted using Liebermann-Burchard reaction. The testing solution was then evaporated in porcelain cup. The resulting residue was dissolved using chloroform in order to dissolve both the steroid and the triterpenoid because the chloroform had the polarity equal to that of the steroid and the triterpenoid. And then anhydrous acetic acid was added to produce acetyl derivative [10]. Concentrated sulfate acid was added through flask wall to hydrolyzed water that would react to the acetate derivative that formed red, brown, or purple or bluish green solution. If brownish or violet ring formed in the border of the solution, it was indicative of the presence of the triterpenoid, while if greenish blue ring formed, it was indicative of the presence of the steroid [11].

In the steroid test the triterpenoid in the ethanol extract of the roots of the *C. tomentosa* produced brownish ring. It indicated that the compound contained the triterpenoid. The triterpenoid compound could be extracted in the ethanol solution and the compound in the group was proven to have antiplasmodium activity *in vitro.* The antimalarial activity of *B. javanica* was caused by the presence of quassinoid. The quassinoid was oxygenated terpenoid that inhibited protein synthesis in malaria parasite [12]. In addition to the quassionoid, it was also caused by the presence of alkaloid indol chantin-6-on, though its activity was weaker than that of the quassinoid [13; 14].

CONCLUSIONS

The *in vitro* antiplasmodial activities of ethanol extracts of *C. tomentosa* roots was categorized to be very active based on the IC_{50} value 2.457 µg/mL.

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