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Research Article

In -vitro Antiplasmodial Activity and the Chromatogram Profile of Active fraction of Central Borneo-Type *Angiopteris evecta* Tubers

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Abstract: *Angiopteris evecta* plant is one of the plants used by people in Central Borneo as antimalarial drug. The study conducted isabout in vitro antiplasmodial activity against the ethanolic extract of *A. evecta* tubers with the IC_{50} value of 2.858±0.27µg/mL.The results of the study showed that *A. evecta* tubers have potential in vitro antiplasmodial activity. Therefore, further study should be conducted to make the fractionation of *A. evecta tubers* and determine the active fractions of *A. evecta* tubers as in vitro antiplasmodium. In vitro antiplasmodial activityis based on Candlejar methods on the active fractions of *A. evecta tubers* using the culture of *P. falciparum*. The results of the fractionation include three fractions, namely FA, FB, and FC. The in vitro antiplasmodial activities ofFA, FB, and FC are expressed ininhibitory concentration of 50%. The results of probit analysisfrom the triplet tests indicate that the IC_{50} mean values of FA, FB, and FC were 37.93±1.19; 3.35±0.07; and>250µg/mL, respectively. Based on in vitro antiplasmodial activity,those of FA were categorized as active, of FB were very potential, and of FC were inactive. The three fractions show that the strongest in vitro antiplasmodial activity was the activity of FB.
Keywords: *Angiopteris evecta*, antiplasmodial, in vitro, *Plasmodium falciparum*, FCR3

INTRODUCTION

The use of natural materials in the treatment is usually based on empirical experiences from generation to generation based on information from ancestors. A study is necessary to find out and explain scientifically the activity of plant as an antimalaria drug. A study on the testing of in vitro antiplasmodial activityis anpreliminary study towards in vivo antiplasmodium assay and clinical assay for malaria drug discovery.

Some plants have been explored such as *B. javanica* as an antimalarial drug because it contains quasinoide. Quasinoide is oxygen ased terpenoids which inhibit protein synthesis in the malaria parasite [1]. In addition to quasinoide, it is also caused by the presence of indole alkaloids*Canthin-6-on*, although its activity is lower than quasinoide [2-3].

Exploration was also conducted by Prozesky *et al*. [4] 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 at50µg/mL. The strongest antiplasmodial activity is in the extracts of dichloromethane for *Ozoroaengleri* and *Balanitesmaughamii* with the same inhibition (IC_{50}1.7µg/mL).

A study conducted by us is to test in vitro antiplasmodial activity against the ethanolic extract of *A. evecta* tubers with IC_{50}value of 2.858±0.27µg/mL (unpublished). The results of the study showed that *A. evecta tubers* potentially have in vitro antiplasmodial activity. Therefore, further studies are necessary to make the fractionation of *A. evecta tubers* andto testthe antiplasmodial activityof *A. evecta tubers* to fractions obtained. Publicationon the recent study has never beenmade and,based onthe search for literatures, no publication on the same study was found. This study contributes in search of *A. evecta tubers*with in vitro antiplasmodial activity to discover a new anti-malarial drug.

EXPERIMENT

Materials

The materials used for the fractionation were n-hexane, ethyl acetate, ethanol and methanol. RPMI, HEPES, $NaHCO_3$, gentamicin, RBC(Red Blood Cell) of blood group O, *P .falciparum* strain FCR3, 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 (28 mL of glycerol; 72mL of 4,2% sorbitol in 0.9%NaCl), Giemsa, and oil immersion were used to test in vitro antiplasmodial activity.

Subjects of the Study

The materials tested were the fractions of *A. evecta* tubers. The parasites used were *P.falciparum* strain FCR3 from the Laboratory of Pharmacology, the Faculty of Medicine, Gadjah Mada University.

Plant Determination and Ethical Clearance

Medicinal plant were sampled in January 2012 from Palangkaraya in Central Borneo and were identified by comparison with authentic specimens at the Herbarium Bogoriense, Research Center for Biology, Indonesian Institute of Science. A voucher specimen is kept in Study Programe of Pharmacy, Faculty of Mathematics and Natural Sciences, Lambung Mangkurat University, South Borneo, Indonesia with number 2296/IPH.1.02/If.8/IX/2012.

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

Trituration

The ethanolic extract of 20g added by the solvent of n-hexane, stirred to be homogeneous,then separated between residue and sediment. The residue was collected and evaporated to obtain n-hexane fraction (FA). The sediment was added the solvent of ethyl acetate, stirred to be homogeneous, then separated between residue and sediment. The residue was collected and evaporated to obtain the soluble fraction of ethyl acetate(FB). The insoluble sediment of ethylacetate was called the insoluble fraction of ethyl acetate (FC). The n-hexane (FA), FB and FC fractions were prepared to test in vitro antiplasmodial activity. Fractions selected and then isolated wasthose with thestrongest in vitro antiplasmodial activity.

In vitro antiplasmodial activity test

The in vitro antiplasmodial activity test of ethanol extracts was conducted by a candle jar method [5-6].

The materials used were weighed and added 100 µL of DMSO and 900 µL of RPMI solution. They were then sterilized through filtration using 0.20 µm of membrane filter. The fractionated materials were ranked in concentrations of 250, 50, 25, 5, 0.5 µg/mL. Chloroquine was used as the positive control in concentration ranks of 40, 20, 16, 12, and 8 ng/mL.

The materials and Plasmodium were prepared. The following steps were as follows: to provide a microplate (96-well) for the test; to add into the microplate 100 µL of RPMI as the negative control, 100 µL of test solution, and chloroquine as the positive control; to add the 100 µL of Plasmodium (the result of synchronization) into the microplate already containing the negative control, the test solution, and the positive control; to place the microplate in the candle jar and to incubate it at a temperature of 37^oC for 72 hours; to take out the microplate from the candle jar after the incubation period was over, and the harvest was done by moving the mixture from each hole into micro tube, which was then centrifuged. The supernatant was discarded.

The smear of the cells (residue) was placed on the slide and fixed with methanol after it got dry. The dry smear was painted with dye of Giemsa 5%, let stand for 30 minutes, and washed with the flowing water. It was left to dry and immersion oil was added. Through the microscope, the number of erythrocytes and parasitaemia of the smear could be seen and counted. The percentage of parasitaemia was calculated by comparing the number of the infected erythrocytes out of 1000 erythrocytes, by employing the formula below:

$$\%Parasitaemia = \frac{\sum InfectedErythrocites}{\sum Erythrocites} x100\%$$

Data of the percentage of parasitaemiaafter treatment at each concentration of the test compound was compared to the percentage of parasitaemia of negative control in order to obtain the parasite growth inhibition (the percentage of inhibition), with the formula:

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

The data obtained was shown in a curve of relationship between the concentration of compound and the percentage of parasite growth inhibition. The IC_{50}value was determined by probit analysis on the percent inhibition with the logarithm of test concentration.

Statistical analysis

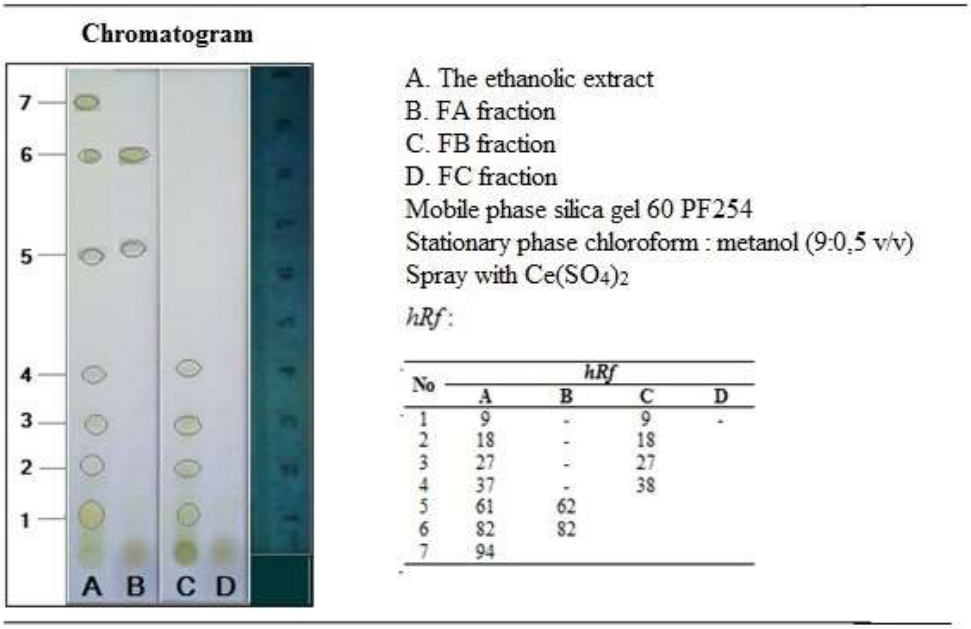
The in vitro antiplasmodial activity test were executed in triplicate and the data is presented as mean ± SEM in the results. The IC_{50}value was determined by

ProbitAnalysis(95 % confidence interval) on the percent inhibition with the logarithm of test concentration using SPSS version 16 for windows. The data IC_{50} value followed analysis with one way ANOVA at 5 % level of significance.

RESULTS AND DISCUSSION

Trituration by using the solvent of n-hexane and ethyl acetate is to separate the chemical compounds contained in the ethanolic extract of *A. evecta tubers*. The monitoring was performed by usinga thin layer chromatography to compare the chromatogram profiles of each fraction in the thin layer chromatography plate (Figure 1). The chromatogram profile of n-hexane fraction(FA) was different fromthat of the soluble fractionof ethylacetate (FB) andthat of the insoluble fractionof acetate (FC). Spots in the chromatogram of FA have different HR ffrom those of FB. In view of polarity, the solvents of n-hexane and ethyl acetatehave the differentlevel of polarity. Polarity of the solvent of n-hexane was lower than that of ethylacetate [7], so the ability to dissolve the chemical compounds in the ethanolic extract of *A. evecta tubers* was different also. The chromatogram profiles showed a good separation. Chemical compounds found in FA were different from those in FB, as well as in FC. No same spot was found in both fractions.

In vitro antiplasmodial activity in the three fractions of FA, FB, and FC were tested consecutively after ensuring that all the three fractions contain different compounds. In vitro antiplasmodial activityin the fractions from the trituration was tested at doses of 0.5; 5; 25; 50; and250usingan incubation period of 72hours.



No	hRf			
	A	B	C	D
1	9	-	9	-
2	18	-	18	
3	27	-	27	
4	37	-	38	
5	61	62		
6	82	82		
7	94			

Fig-1: Profile TLC of the ethanolic extract, FA, FB, and FC fractions of *A. evecta* tuber

The resultsof the tests for in vitro antiplasmodial activityin the fractions show the percentage of parasitemia. The percentage of parasitemia was inverselyproportional to the increasee of concentration(Table 1).

Table-1: The percentage of parasitemia an incubation period of 72hours

Fraction	Consentration (µg/mL)				
	250	**50**	**25**	**5**	**0,5**
FA	1,108±0,25	8,124±0,35	10,890±0,04	11,398±0,22	12,235±0,03
FB	0,062±0,05	2,159±0,05	6,498±0,12	7,236±0,06	9,424±0,07
FC	10,151±0,05	10,296±0,02	10,625±0,03	11,951±0,03	12,329±0,05
K(-)	14,627±0,63				
K(+)	**$40x10^3$**	**$20x10^3$**	**$16x10^3$**	**$12x10^3$**	**$8x10^3$**
Kloroquin	0.000±0.00	0.080±0.14	0.290±0.50	0.368±0.51	0.547±0.62

From the percentage of parasitemia and negative control, the percentage of inhibition in *P. falciparum* growth was calculated. The percentage of inhibition in growth of *P. falciparum* after the fractions were given showed a greater value with increasing concentration. In FA, the mean percentages of inhibition were 16.316±0.267; 22.080±1.565; 25.549±0.313; 44.459±2.398; 92.426±1.722,

respectively; in FB, 35.575±0.514; 50.529±0.465; 55.575±0.847; 85.245±0.366; 99.574±0.370, respectively; and in FC, 15.711±0.346FC; 18.298±0.210; 27.359±0.258; 29.615±0.184; 30.603±0.381, respectively (Figure 2).

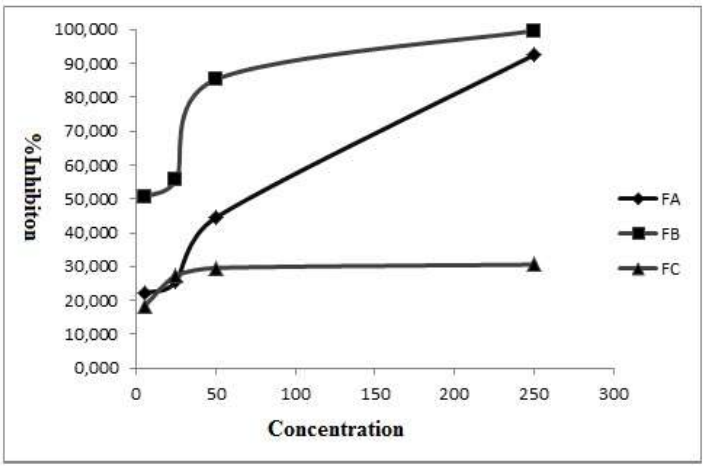


Fig-2: Relationship of the percent inhibition with the logarithm of test concentrationof FA, FB, FC fractions

The results of the test for in vitro antiplasmodial activity in FA, FB, and FC show the mean percentage of parasitemia, the mean percentage of inhibition, and IC_{50}values (Table 1). The percentage of parasitemia was smaller with the larger doses of all the treatments. In vitro antiplasmodial activityin the three fractions(FA, FB, FC) was expressed in IC_{50} using a probit analysis with SPSS10.0, while the analysis of the doses of treatment used the percentage of inhibition in growth of *P. falciparum*. The results of the probit analysis from the triplets show that the mean IC_{50} values of FA, FB, FC, and Chloroquine were 37.93±1.19; 3.35±0.07; >250; and $4.807x10^{-3}$±0.10µg/mL, respectively. ANOVA analysis results significant difference of three fractions and Chloroquine. It shows that the three fractions have different of in vitro antiplasmodial activity, as well as a significant difference to Chloroquine.

The smallest IC_{50}value shows that in vitro antiplasmodial activity was stronger.The extract was stated active if the IC_{50}value was <5 µg/mL and moderately active if the IC_{50} ranged from 5-50µg/mL [8], very potential if the IC_{50}value was <1-4.9 µg/mL [9], very good if the IC_{50}value was<5 µg/mL [10]. Based on the results, in vitro antiplasmodial activity in FA was moderately active, in FB very potential, and in FC inactive. Of the three fractions, the strongest in vitro antiplasmodial activity was FB.

CONCLUSION

The in vitro antiplasmodial activity in ethyl acetate fraction(FB)were very potential, in n-hexane fraction(FA) was potential and in fraction insoluble ethylacetate (FC) was not potential. Of the three fractions, the strongest in vitro antiplasmodial activity was FB. The compound group of the active fractions of FBwas aglycoside.

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