Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2018, 10(11): 41-47

Research Article

ISSN : 0975-7384
CODEN(USA): JCPRC5

The Activity of Anti-Plasmodium of Alkaloid Fraction from Bark Yellow Rope (*Archangelsia flava* (L) *Merr*)

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ABSTRACT

Malaria is a disease caused by Plasmodium spp normally transmitted by mosquito Anopheles spp. This disease became known globally and has attracted attention from national and international because it can cause death, especially for infants, toddlers and pregnant mother. However, an endemic plant Papua that is used as anti-malaria, is Archangelsia flava.Merr. This study was aimed to determine the activity value of anti-plasmodium alkaloids fraction using methanol extract of bark Archangelsia flava.Merr against Plasmodium falcifanrum during 48 hours of in vitro incubation. Alkaloid fraction obtained from the separation of the methanol extract using liquid-liquid extraction with various solvents of acidic and alkaline. Plasmodium culture was applied chandel jar method meanwhile anti-plasmodium activity test was conducted using in vitro. The activity of anti-plasmodium stated through Inhibition Concentration (IC50) value which known to inhibit 50% growth of Plasmodium. The results showed that value of IC50 throughout 48 hours of culturing process was 0.051 µg /mL. Therefore, it is concluded that the alkaloids fraction of Archangelsia flava Merr bark contained anti-plasmodium activity and has performed excellently during 48 hours of culturing process.

Keywords: Activity; Anti-plasmodium; Alkaloid fraction; *Archangelsia flava Merr*; Papua

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INTRODUCTION

Malaria is a disease caused by *Plasmodium* spp. normally transmitted by mosquito *Anopheles* spp. This disease became known globally and has attracted attention from national and international because it can cause death, especially for infants, toddlers and pregnant mother. In addition to that malaria causes anaemia and can decrease work productivity of human. From Indonesian Health Service data in 2010 obtained that the number of people throughout Indonesia has reached 211.677 cases, and the highest number of Extraordinary Events were found in Kalimantan, East Nusa Tenggara, Papua and West Papua [1]. Furthermore, in 2010, prevalence rate of malaria has increased tremendously compared to 2007, from 2.85% to 10.6% and in 2012 the number increased sharply to 417 819 cases corresponding to 97.4% from previous years [2].

Despite the worldwide decline in the numbers of malaria cases, however, from the distribution finding of this disease especially in India and Indonesia among other Asian countries, therefore, special considerations are still in need to prevent the further distribution and to cure this disease. The biggest obstacle to the Elimination of malaria,

according to World Malaria Report 2017 was due to the existence of resistant parasite to antimalarial drugs and resistance mosquitoes against insecticides. Therefore, it is crucial to look for new alternative antimalarial medicine that can effectively cure from local plants [3,4]. The utilization of the local plants as an herbal health support in both prevention and treatment in ethnic/medicinal plants has long been conducted by ancestors or our ancestors. One of the plants that are frequently used by the indigenous people of Papua starting from Sorong to Jayapura is yellow rope plant (*Arhangelsia flava Merr*) which in the local language of the tribe Moy Kempton called Tanggang Yanggu. Specifically, Moy Tribe has been using yellow root plant (*Arcangelisia flava Merr*) as a traditional medicine plant. The society believes the extracts bark of this plant has a very good effect for the body, because it can help to protect malaria, reduce the fatigues, and to maintain overall body health [5]. Traditionally bark of this plant were taken then boiled in water, followed by removing the leftover plants and the water is ready to consume to treat malaria and some other particular pains [6].

Scientific study of this plant has been reported about the presence of the alkaloid content of the bark of yellow rope and rods are activities as anti-hepatitis [7]. In addition methanol extracts from bark of this plant has a value of cytotoxic LC50 of 89.5 µg/mL against larvae of shrimp Artemia salina Leach [8]. Furthermore, from the ethyl acetate extract method, bark of this plant has showed anti-plasmodium activity corresponding to IC50 values of 1.32 µg/mL.

In this research, anti-plasmodium activity of alkaloids fraction of methanol extract from bark yellow plant rope using jar chandel and anti-plasmodium activities carried out by the method in vitro has been investigated. Activities anti-plasmodium stated at the Inhibition Concentration (IC_{50}) corresponding to 50% inhibition growth of Plasmodium has been reached.

MATERIALS AND METHODS

Plant Materials

Samples of yellow rope plant (*Archangelsia Flava Merr*) obtained from the forest village in Maribu, District of West Sentani, Jayapura Regency, Papua Province. Total ten kilograms of samples have been stored in chemistry laboratory of Universitas Cenderawasih for further uses.

Chemicals used in this research were determined as pure for analysis and some additional second grade chemicals. Specifically, organic solvent methanol, dichloromethane, hydrochloric acid, ammonium hydroxide, TLC plate (silica gel GF254-coated plate and ODS) and ODS silica gel G60. Other additional are namely: iron (III) chloride 1%, reagent Meyer, Wagner reagents, reagent Dragendroff, and acetone.

The equipment used in this study include analytical balance, a set of maceration, R-200 rotary evaporator Buchi, chamber TLC, column chromatography, sample container bottle, and some glassware commonly used laboratory for preparation.

Simplicia Powder Making

In this process, bark of yellow plant rope collected and cleaned by washing with water followed by cutting into smaller pieces and applied sun drying. After that, these pieces were blended to homogenize the size into powder

form were then called as simplicia powder. This powder were then measured about 500 grams to be used for maceration.

Extraction and Isolation

500 grams of simplicia powder prepared were macerated used 1.5 L of methanol solvent for duration of 2×24 hours. Methanol extract was condensed using rotary evaporator at temperature of 40°C. From this evaporation procedure, condensed methanol extract obtained was 3.565 gram was then mixed with hydrochloric acid 1% until it reached pH of 2.6. Extraction product was parted using dichloromethane water by applying separating funnel to separate undissolved organic compound. Acid phase of water has used ammonium hydroxide to achieve pH level of 9-10 followed by the separation of dichloromethane base and phase of water base. Phase of dichloromethane base were condensed and later mixed with methanol to be separated using High Performance Liquid Chromatography (HPLC).

Fractionation using High Performance Liquid Chromatography

Column Chromatography was done using stationary phase of Silica Gel G60 with size range of 70-230 mesh; meanwhile for mobile phase methanol and chloroform were used. The fractionation results then identified and purified using high performance liquid chromatography.

Identification of Active Compound using Chromatography

In order to identity the profile of alkaloid compounds from isolation product, Thin Layer Chromatography was applied using solvent of methanol: chloroform (3:2).

Activity of Anti-plasmodium Test

Anti-plasmodium test has applied the method of in vitro using 3D7 *Plasmodium falciparum* strains in which were found sensitive to chloroquine. Activity test is done by dissolving the test substance in DMSO and then made serial dilution in RPMI medium to obtain a final concentration of 100 µg/ml, 10 µg/ml, 1 µg/ml, 0.1 µg/ml and 0.01 µg/ml, respectively. Furthermore, inside the test solution tube a suspension of parasites was added with parasitemia levels of ± 1% and 5% hematocrit. The cultures were incubated for 48 h at 37°C. Cultures were then harvested and made for preparations blood thinner layer with 20% Giemsa staining. Lastly, percent of parasitemia was calculated and the percent inhibition of growth of *P. falciparum* was calculated by counting the number of infected erythrocytes per 1000 erythrocytes under the microscope observation.

RESULT AND DISCUSSION

Preliminary Test

Preliminary test of phytochemical screening was performed to determine the profile of the active compound contained in the methanol extract of the bark of the yellow rope (Figure 1). Results of phytochemical using screening reagent Meyer, reagents Wagner, and reagents Dragendroff, from the methanol extract of the bark of yellow string containing alkaloids has showed positive results for each reagent added marked with formation of deposits brown to yellow (Table 1). It is caused by a reaction that occurs between nitrogen atoms in the alkaloids and metals from reagents resulted in form of complex compounds [9].

Table 1. Qualitative test of alkaloids from Bark of the yellow rope

Reagents	Results
Mayer	+ (purple sediment)
Dragendroff	+ (dark brown sediment)
Wagner	+ (bright yellow sediment)

Note: (+): alkaloid content

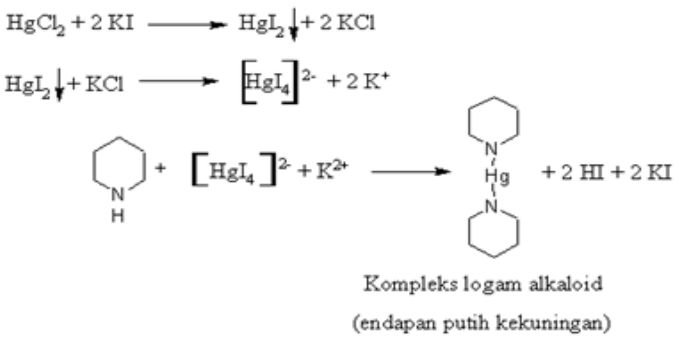

Figure 1. Reaction alkaloid with Dragendroff reagent

Alkaloid Isolation

500 grams of simplicia powder prepared was macerated using 1.5 L of methanol solvent for 2 × 24 hours. Result from methanol extraction was condensed using rotary evaporator at 400C. Product obtained from evaporator was condensed methanol extract at around 3.565 grams. Later on pH was adjusted to be 2.6 by the addition of 1% hydrochloric acid. Furthermore, extract product was parted using dichloromethane and water. This step was aimed to remove the excess of organic acids and other undissolved organic compounds that are being distributed into organic dichloromethane. Alkaloid salt itself will be distributed in the phase aqueous acidic. Acidic aqueous phase containing alkaloids was tested using Dragendroff reagent and some other alkaloids. The test results showed this fraction does not contain alkaloids, therefore this fraction was divided using a base (ammonium hydroxide) to reach pH of 9.8. Fraction of water was being parted using dichloromethane in order to be remove alkaloid extracted into dichloromethane phase base phase. Meanwhile, salt ammonium chloride formed will be extracted into the water phase. The existence of alkaloids tested using Dragendroff reagents and test results showed dark brown sediment is formed, followed by concentrating fraction of dichloromethane resulted in 0.2325 g (0.046%) from 500 g sample. Final alkaloid product was further being purified using column chromatography (CC), and high performance liquid chromatography (HPLC).

The Activity of Anti-plasmodium Fraction of Alkaloids

The test of anti-plasmodium is carried by using in vitro methods. This testing was performed against the growth of the parasite *plasmodium falciparum* using concentrations of 1, 0, 100,10 1dan 0.01 µg/mL in the media called RPMI (Roswell Park Memorial Institute) and the process was incubated for 48 hours at 37°C. On the solution prepared suspension of parasites were added with levels of parasitemia ± 1% and 5% hematocrit. These preparations were incubated for 48 hours at a temperature of 37°C and thin layer of blood preparations were made with giemsa staining of 20% in order to simplify observing the parasite under a microscope. Further observation showed growth

inhibition of *Plasmodium falciparum* by counting the number of infected erythrocytes per 1000 erythrocytes. The results for Antimalarial activity testing of *Plasmodium falciparum* with extract of yellow rope (*Arcangelisia flava Merr*) are presented in table 2 below.

Table 2. Results Activity Test of *Plasmodium falciparum* Growth

Dosages (µg/ml)		Replicate	% Parasitemia		% Growth	% Inhibition	% Average Inhibition
			0 h	48 h			
Control (-)		1	1.00	4.78	3.78	-	-
		2	1.00	4.86	3.86	-	
100		1	1.00	1.30	0.30	92.06	90.85
		2	1.00	1.40	0.40	89.64	
10		1	1.00	1.60	0.60	84.13	84.94
		2	1.00	1.55	0.55	85.75	
1		1	1.00	2.09	1.09	71.16	70.30
		2	1.00	2.18	1.18	69.43	
0.1		1	1.00	2.76	1.76	53.44	54.05
		2	1.00	2.75	1.75	54.66	
0.01		1	1.00	3.30	2.30	39.15	38.62
		2	1.00	3.39	2.39	38.08	

Table 2 presented above showed a significant data of antimalarial activity from *Plasmodium falciparum*. This suggests that there is significant correlation between the concentration of the samples and the percentage of inhibitors. The higher dosage concentration percentage value then the higher inhibition was observed. By linking the value of the % resistance/inhibition and log concentration IC_{50} value was calculated.

From the results presented in the table for alkaloid fraction of bark yellow rope with concentrations between 0.01-0.1 µg/mL extract yellow ropes can inhibit the growth of parasites as much as 38.62-54.05%. Or in other words the concentration range of alkaloid extract of bark yellow rope mentioned owns 50% potential to inhibit the growth of parasites. Based on the data presented at table 2, therefore the analysis done to measure the effect value of test concentration against the inhibition using log concentration to determine IC_{50} value.

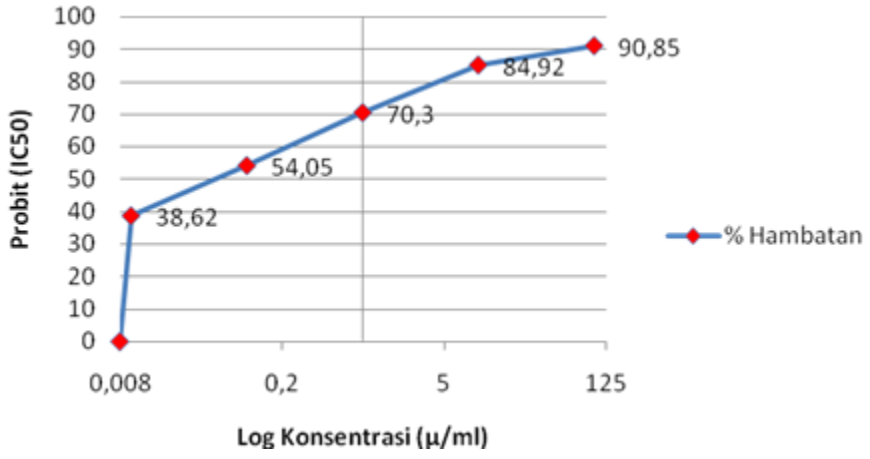

Figure 2. Prohibit vs Log Concentration

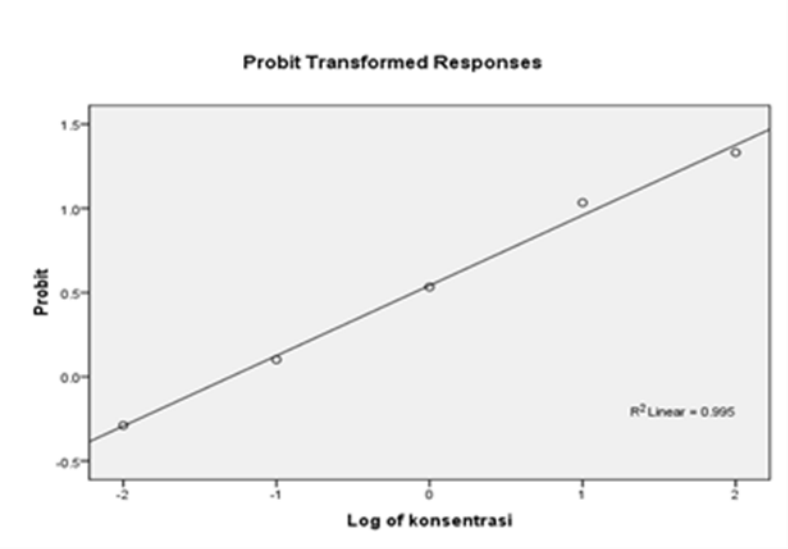

Figure 3. Prohibit vs Log Concentration to determine IC_{50} value

Based on the Figures 2 and 3 the relationship between percent average inhibition with log concentration as well as the calculation of the value of the regression liner alkaloid fraction rope yellow bark against the parasite plasmodium IC_{50} values obtained from then fractions of the alkaloid bark rope Yellow is 0.051 µg/ml and categorized very toxic. If it's compared with previous research study of the anti-plasmodium test using ethyl acetate extracts of bark yellow rope (IC_{50} value of 1.32 µg/ml) the values of the IC were referred to the category which stated that the test substance in anti-plasmodium activity in vitro is divided into 3 classes: the test substance with the activities of IC_{50} values are categorized best when ≤10 µg/ml, activity good value IC_{50} 10-50 µg/ml and poor with IC_{50} values of ≥50 µg/ml [10,11].

If it is compared with the reference value of the alkaloid fraction bark with yellow rope corresponding to IC_{50} value of 0.051 anti-plasmodium most excellent activities in Plasmodium incubation at 48 hours, confirmed. When referred to the life cycle of *Plasmodium falciparum*, it is observed that the fraction of alkaloids from bark yellow rope anti-plasmodium activity tend to be on stage merozoites. This is due to the life cycle of *Plasmodium falciparum* in 48 hours or 72 hours of being on stage merozoites [12,13]. Plasmodium activity can be assumed that the presence of alkaloids in these fractions could prevent the transition from merozoites to tropozoit stage.

However, work on stage merozoites alkaloid fraction still in the process of identifying, therefore, other supporting data are required, because finding from this research was only carried out by in vitro method. To determine the ability activity more researches required to be performed, such as in vivo tests when system isolates metabolites in the body. Hence, it is necessary to conduct additional studies to obtain more data.

CONCLUSION

Alkaloid fraction using methanol extract of the bark of yellow rope has activity as anti-plasmodium with the best working performance was obtained at 48 hours of culture with IC50 value of 0.051 µg/ml.

ACKNOWLEDGEMENTS

On this occasion we would like to express our gratitude to several parties who have helped throughout the process

1. Ministry of higher education, Research and Technology for funding

2. Laboratory Herbal Medicine Surabaya on the UNAIR for collaboration work during anti-plasmodium analysis

3. Head laboratory of chemistry department FMIPA UNCEN for screening test

4. Mr Septinus Atatu villager from Maribu who have helped in the sample collection from the forest village in Maribu, District of West Sentani, Jayapura Regency, Papua Province.

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