

Addendum

In vitro antimalarial activity of Jaloh leaves extract on *Plasmodium falciparum*

Nuzul Asmilia¹, Amalia Sutriana², Erdiansyah Rahmi³, Sugito¹

¹Laboratory of Clinic, Veterinary Medicine Faculty, Syiah Kuala University; ²Laboratory of Pharmacology, Veterinary Medicine Faculty, Syiah Kuala University; ³Laboratory of Hystology, Veterinary Medicine Faculty, Syiah Kuala University. Corresponding author: nuzulasmilia@yahoo.com

Abstract. Malaria still cause health problem in tropics including Indonesia, since its generate high mortality rate. Medication of Malaria has been performed long time ago without optimum result. One reason is the high rate of *P.falciparum* resistance on conventional antimalaria drug. This research aim to evaluate the activity of jaloh leaves extract (Willow leaves) on the inhibition of *P.falciparum* in vitro. This was a laboratory experimental research that using Indonesian isolates of cloroquin resistance *P.falciparum* obtained from NAMRU-2, Jakarta. *P.falciparum* isolate that has been synchronized were divided in to 3 groups of treatment : Jaloh leaves ethanolic extract, jaloh leaves ethyl acetate extract, and jaloh leaves n-hexane extract. Each group were then allotted into 5 dosage levels: 6,25ug/ml; 12,50ug/ml; 25,00ug/ml; 50,00ug/ml; and 100ug/ml. The observation of parasitemia degree were done 48 hours post-incubation in which the thick blood smear were colored with Giemsa and then examined using biocular microscope. Data were analyzed using one way ANOVA and Newman-Keuls test. The result showed that the percentage of *P.falciparum* growth inhibition were sinificantly different ($P < 0.05$) among treatments wherein ethanol extract and ethyl acetate extract more inhibit *P.falciparum* growth than the hexane extract. The administration of different dosage also resulted in significance different ($P < 0.05$) of *P.falciparum* growth inhibition. This research concludes that jaloh leaves extract could inhibit *P.falciparum* growth.

Key words: Malaria, *Plasmodium falciparum* , jaloh,

Introduction

Malaria is one of complex infectious disease in this era and known as a major cause of high morbidity and mortality in the world . WHO reported that about 490 million people suffered malaria in 2002 with mortality rate about 1,5-2,7 million people per year (Anonymous, 2002). This disease widespread in 90 % Africa, south America, and Asia including Indonesia. Several effort to prevent this disease has been carried out but its prevalence still high (WHO, 1977:1998).. Its due to several obstacle in eradication effort such as plasmodium resistance on conventional antimalaria drug and mosquito resistance on insecticide. Therefore, some studies related to invention of new drug have to be conducted particularly using traditional plant. People In Aceh, has been recognized a medicinal plant namely Jaloh (Willow tree). This plant is a member of family Salicaceae with species name *Salix tetrasperma* Roxb. Jaloh were traditionally used to treat fever due to malaria. Its traditionally proven in reducing fever in several days after drink jaloh juice (Sugito, 2007). In order to develop jaloh leaves as traditional medicine become fitofarmaka materials, it is need to isolate and identify the active component of Jaloh leaves and also to find out active component which has good inhibition on the *P. falcifarum* growth. In turn, it is expected that chemical structure of antimalaria from jaloh extract could be developed to increase the efficacy on its pharmacokinetics and pharmacodynamics values as anti malaria drug.

This research was purposed to develop exploration technique of metabolite in Jaloh leaves as antimalaria in society with the particular aim was to determine the active component of Jaloh leaves which has a good parasitemia inhibition in *P. falcifarum* culture.

Material and Methode

Materials

The leaf of jaloh were collected in Darussalam, Banda Aceh, and were identified by comparison with authentic specimens at Biology Laboratory Syiah Kuala University. Chemicals for extraction is jaloh leaves, 70% Ethanol, n-heksan, ethyl acetate (EtOAc), methanol (MeOH), cloroform, ammonia, hydrochloric acid, silica gel 60 (Merck), sterile and Aquabidest leaves jaloh.

Material for culture is *Plasmodium falciparum* isolates the local strain, RPMI 1640 medium (Gibco); Aqua bidestilata (PT. Ikapharmindo Son Mas), solution of sodium bicarbonate, HEPES solution (Merck), preparation of erythrocytes, human serum, the patient's blood, D - sarbitol 5% and aquadest, NaOH, anti-coagulant Citrat Phospat Dextrosa (CPD), Aquadest sterile, Buffer, Giemsa, oil and emersi *Plasmodium falciparum* isolates sensitive and resistant local strains kloroquin obtained from NAMRU-2 Jakarta.

Plant fraction preparation

The jaloh leaves was air dried and powdered. The extracts then prepared by maceration the powder with ethanol 70% at room temperature for 24 hours. The proses was repeated three times. Extracts of cotton and filtered using filter paper, and then obtained the filtrate collected and evaporated using a rotary evaporator with water bath and vacuum pump. Furthermore jaloh leaf crude extracts was fraction using heksan solution and residues partitioned with Ethyl Acetate. Then the separation hexan solution, obtained by ethyl acetate is evaporated with a rotary evaporator using a vacuum pump.

In Vitro antiplasmodial activity testing

A strain of *P. falciparum* chloroquine resistant strain was used in the study. Parasited were cultured continuously according to Tragger and Jensen (1976).

The parasites were maintained in vitro in human red blood cell (O) diluted to 1 % hematocrit in RPMI 1640 medium, supplemented with 7,68 mM Hepes and 23,78 mM NaHCO₃, and complemented with 10% human O serum and distributed into wells of a 96-well micro plate (100µl per well). Before using, parasite cultures were synchronized by a D-sorbitol lysis in order to obtain ring stage of *P. falciparum* as reported by Lambros and Vanderberg (1979).

Jaloh leaves extract at 3 groups of treatment (ethanol extract, ethyl acetate extract, and n-hexane extrat) with 5 dosage level (6,25 ug/ml; 12,50 ug/ml; 25,00 ug/ml; 50,00 ug/ml; and 100,00 ug/ml). were added into wells. The microplate containing parasite culture and jaloh leaves extract was the incubated in CO₂ incubator at 37°C candle jar incubator for 48 hours. After that blood film were taken and level of parasitemia determined on Giemsa stained smears by counting 200 erythrocyte. The observation of parasite growth degree was counted under microscope 10 X 100.

The data were analyzed using ANOVA and then followed by Newman-Keuls test to evaluate the differences among the treatment.

Results and Discussion

The result of phytochemical screening test of jaloh leaves extract showed that this extract, positively, consist of alkaloid and saponin. The result showed that the percentage of *P. falciparum* growth inhibition were significantly different ($P < 0.05$) among treatments wherein ethanol extract and ethyl acetate extract more inhibit *P. falcifarum* growth than the hexane extract. The administration of different dosage also resulted in significance different ($P < 0.05$) of *P. falciparum* growth inhibition. This research concludes that jaloh leaves extract could inhibit *P. falciparum* growth (Table 1)

Tabel 1. The average percentage (\pm SD) of *P. Falciparum* growth inhibition at various treatment groups after 48 hours cultured

Doses (μ g/ml)	<i>Plasmodium falciparum</i> growth					
	Ethanol extract		ethyl Acetat extract		n-heksan extract	
	% Parasitemia	% Inhibition	% Parasitemia	% Inhibition	% Parasitemia	% Inhibition
control	24		24		31	
6,25	14,5	39,58	15	37,5	19,5	19,35
12,50	13	45,83	12	50,0	25	37,09
25,00	11,5	52,08	11	54,0	19	38,70
50,00	8,5	64,58	11	54,0	19	38,70
100,00	8,5	64,58	8	66.66	19	38,70
Average		53,33A		52,43A		34,51B

Note: Different letter superscript at same line show very real ($p < 0,05$)

Conclusion

In conclusion, the present study showed that ethanol extract, ethyl acetate extract of *Salix tetrasperma* leaves displayed better antiplasmodial activity invitro than n-hexane extract.

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