

## In Vitro Antimalarial Activity of Methanolic Extract of *Morus Alba* L. Leaves against *Plasmodium Falciparum* 3D7

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### Abstract

Malaria is one of major global health problem, especially in sub-tropical and tropical countries. The increasing incidence of parasite resistance to recent antimalarial drug encourage any efforts in antimalarial drug discovery. Plants and traditional medicines are an important resource for the discovery and development of new antimalarial agents. *Morus alba* L. (Moraceae), commonly known as "murbei", has been traditionally used to treat fever and malaria. The aim of this research was to investigate antimalarial activity of methanolic extract of *M. alba* L. leaves against *Plasmodium falciparum* 3D7 in vitro. Dried leaves of *M. alba* L. was extracted with methanol yielded methanolic extract, followed by in vitro antimalarial activity assay. The result was analyzed by Probit Analysis. Phytochemical screening showed the presence of triterpenoids, flavonoids and glycosides. Result of this study revealed that methanolic extract of *M. alba* L. leaves was active against *P. falciparum* 3D7 in vitro, with an  $IC_{50}$  value of 0.02  $\mu$ g/mL.

**Keywords:** *Morus alba* L., methanolic extract, *Plasmodium falciparum* 3D7, antimalarial

### INTRODUCTION

Malaria is an infectious diseases caused by Protozoa from Genus *Plasmodium*, passed through female *Anopheles* mosquito (Gandahusada, S. et al, 2004). Malaria still remains one of the most important global public health problem and mortality from malaria appears to be increasing in the highest risk groups, children and pregnant women. A major problem in global malaria control program is the increasing incidence of resistance to current antimalarial drugs (Bloland, P.B. 2001). Therefore, any efforts in antimalarial drugs discovery and development are urgently required.

Natural products is one of resource that provide lead compounds with various pharmacological activities. The first anti-malarial drug was quinine, isolated from the bark of *Cinchona* species (Rubiaceae) in 1820 which is traditionally used to treat fever. The first line antimalarial drug nowadays, artemisinin, also isolated from herb *Artemisia annua* (Compositae) which is traditionally used in China to treat fever (Saxena, S. et al, 2003). Historical background of quinine and artemisinin isolation has encouraged any investigation on plants based-ethnopharmacological approach for its antimalarial activity.

*Morus alba* L. leaves belongs to family Moraceae, in Indonesia commonly

known as “Murbei” is traditionally used to treat fever and malaria. *M. alba* L. is widely distributed in Indonesia (Departemen Kesehatan RI, 2000). *M. alba* L. leaves has been proved for its antioxidant activity (Nikkhah, E. et al, 2009), adaptogenic (Nade, V.S. et al, 2009), immunomodulatory (Bharani, S.E.R. et al, 2010) and hepatoprotective activity (Hussein, M.S. et al, 2010). This research aims to investigate *in vitro* antimalarial activity of methanolic extract of *M. alba* L. leaves against *P. falciparum* 3D7.

## **MATERIALS AND METHODS**

### **Plant material**

Leaves of *M. alba* L. was collected from Gianyar Bali, Indonesia, in Pebruary 2011. A voucher specimen was identified at the Eka Karya Bedugul Botanical Garden, Tabanan Bali, Indonesia.

### **Parasite *P. falciparum* 3D7**

*P. falciparum* strain 3D7 obtained from Institute of Tropical Disease, Surabaya

### **Chemicals and reagents**

All reagents were analytical grade, RPMI 1640 medium (Gibco), Giemsa (Merck), HEPES, hypoxanthine, gentamycin, phosphate buffer, bicarbonate sodium, Immersion oil (Merck) and dimethyl sulfoxide (DMSO).

### **Equipments**

Glassware, vaccum rotary evaporator, 24-microwell plate, CO<sub>2</sub> incubator, laminar air flow

### **Procedure**

**Extraction.** Three hundreds gram of coarsely powder of *M. alba* L. dried leaves was extracted with MeOH at room temperature overnight. The solvent was evaporated, yielded 59.20 g of crude extract.

**Phytochemical screening.** Phytochemical screening for the presence of alkaloids, terpenoids, steroids, saponins, tannin, glycoside and flavonoids was conducted using methods previously

described (Departemen Kesehatan RI, 1989; Evans, W.C., 2000; Hakim et al., 2010; Jones, W. P. and Kinghorn A. D., 2006)

### **In vitro antimalarial activity assay.**

The antimalarial activity of methanolic extract of *M. alba* L. was determined by the procedure described by Budimulya, A.S. et al (1997). Briefly, methanolic extract of *M. alba* L. was separately dissolved in dimethyl sulfoxide ( $10^{-2}$  mol L<sup>-1</sup>) and kept at -20°C until used. Parasite *P. falciparum* 3D7 was propagated in a 24-well culture plate in the presence of a series concentration (0.01, 0.1, 1, 10, and 100 µg/mL) of *M. alba* L. extract, and incubated for 48 hours. Parasite growth was determined by making a thin blood film fixed with MeOH and stained with Giemsa. The antimalarial activity of extract was expressed as an IC<sub>50</sub> value. IC<sub>50</sub> value was defined as extract concentration at 50% inhibition of parasite growth relative to an untreated control.

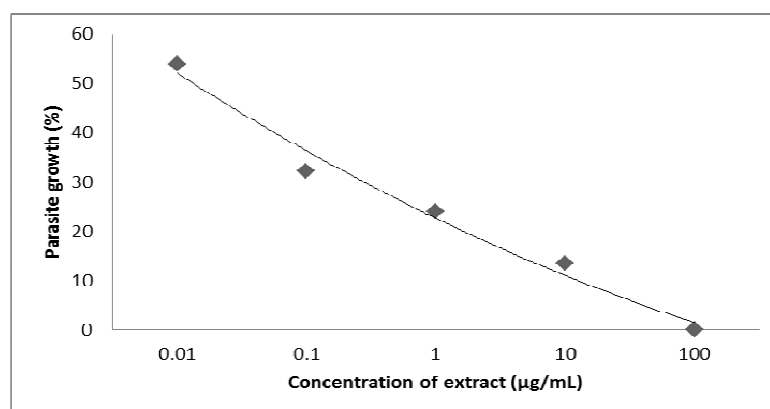
## **RESULTS AND DISCUSSION**

Phytochemical screening of methanolic extract of *M. alba* L. leaves showed the presence of triterpenoids, glycosides and flavonoids. Antimalarial activity assay of the extract was carried out to evaluate the effect of the extract to inhibit parasite growth in red blood cells (schizontocidal effect in erythrocytic phase), which calculated from % parasitemia after 48 hours incubation. During erythrocytic phase, parasite present in several morphological development stages in ring form, trophozoite and schizont (Gandahusada, S. et al, 2004). Parasitemia obtained by counting number of infected red blood cells per 1000 red blood cells (Schlichthele, M. et al, 2000). Data obtained from antimalarial activity assay of this extract are shown in Table 1 and Figure 1. An increase in concentration of methanolic extract lead to a decrease in parasite growth and an increase in percentage inhibition of parasite growth.

**Table 1.** In vitro antimalarial activity of methanolic extract of *M. alba* L. leaves against *P. falciparum* 3D7

Concentration of methanolic extract ( $\mu\text{g/mL}$ )	Result			
	% parasitemia		% Parasite growth	% inhibition
	D <sub>0</sub>	After 48 h		
Untreated control	1.03	3.12	100	-
0.01	1.03	2.16	53.96	46.04
0.1	1.03	1.70	32.13	67.87
1	1.03	1.53	23.98	76.02
10	1.03	1.31	13.39	86.81
100	1.03	0.97	0	100

Notes: D<sub>0</sub> = % parasitemia used for antimalarial assay  
N = 2



**Figure 1.** The growth of parasite *P. falciparum* 3D7 with the presence of series concentration of methanolic extract of *M. alba* L. leaves

Data in Table 1 was then analyzed using Probit analysis for result interpretation. The result showed that methanolic extract of *M. alba* L. could inhibit the growth of parasite *P. falciparum* with an IC<sub>50</sub> value of 0.02  $\mu\text{g/mL}$ . According to Kohler, I. et al (2002), extract was considered to be active and prospective in antimalarial assay in vitro when its IC<sub>50</sub> is < 50  $\mu\text{g/mL}$ . Therefore, methanolic extract of *M. alba* L. leaves

was considered to be active and has promising antimalarial activity. The antimalarial activity of this extract also supports the traditional use of *M. alba* leaves for treatment of malaria. This activity may be due to the presence of triterpenoids and flavonoids in methanolic extract. Saxena, S. et al (2003) reported that several classes of the plant secondary metabolites are responsible for antimalarial activity, including terpenoids and

flavonoids. The most pronounced components from family Moraceae were prenylated flavonoids (Hakim, E.H. et al, 2007). Widyawaruyanti, A. (2007) reported that several prenylated flavonoids, including artocarpone A, artocarpone B, artonin A, cycloheterophyllin, artoindonesianin R, heterophyllin, heteroflavanone C and artoindonesianin A-2, isolated from dichloromethane extract of *Artocarpus champedon* Spreng stem bark (Moraceae) revealed potent antimalarial activity against *P. falciparum*. Several prenylated flavones isolated from the root of *A. altilis* also reported for antimalarial activity (Boonphong, S. et al, 2007). Future research on separation of methanolic extract of *M. alba* L. leaves guided with its antimalarial activity and followed by structure elucidation is needed to obtain active compounds as antimalarial.

## CONCLUSION

Result of this study revealed that methanolic extract of *M. alba* L. was active as antimalarial against *P. falciparum* 3D7 with an IC<sub>50</sub> value of 0.02 µg/mL. Further research is needed to isolate compounds from this extract that have significant contribution for its antimalarial activity using bioactivity guided isolation.

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